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FILE 'LIFESCI' ENTERED AT 16:15:49 ON 11 DEC 2000
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E2            4     LUBITZ W */AU
E3          121 --> LUBITZ WERNER/AU
E4           80     LUBITZ WOLFGANG/AU
E5            1     LUBITZKI LOTHAR/AU
E6            7     LUBITZSCH PETER/AU
E7            6     LUBITZSCH WOLFGANG/AU
E8            1     LUBIVYI V G/AU
E9            1     LUBIW A/AU
E10           1     LUBIZ M/AU
E11           1     LUBJA J/AU
E12           2     LUBJAHN L/AU
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=> s e1-e3

L1 692 ("LUBITZ W"/AU OR "LUBITZ W *"/AU OR "LUBITZ WERNER"/AU)

=> s l1 and fusion protein (5a) bacillus

L2 0 L1 AND FUSION PROTEIN (5A) BACILLUS

=> s l1 and (fusion protein or heterologous protein)

L3 26 L1 AND (FUSION PROTEIN OR HETEROLOGOUS PROTEIN)

=> dup rem l3

PROCESSING COMPLETED FOR L3
L4 12 DUP REM L3 (14 DUPLICATES REMOVED)

=> d bib ab 1-12

L4 ANSWER 1 OF 12 CAPLUS COPYRIGHT 2000 ACS
AN 1999:96508 CAPLUS
DN 130:178339
TI Production of genetically engineered S-layer protein that is secreted into
the periplasm or extracellularly and that can contain integrated proteins for affinity and immuno reactions
IN **Lubitz, Werner**; Resch, Stephanie

PA Austria
SO Ger. Offen., 34 pp.
CODEN: GWXXBX
DT Patent
LA German
FAN.CNT 1

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
PI DE 19732829	A1	19990204	DE 1997-19732829	19970730
WO 9906567	A1	19990211	WO 1998-EP4723	19980727

W: AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GE, GH, GM, HR, HU, ID, IL, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, UA, UG, US, UZ, VN, YU, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM
RW: GH, GM, KE, LS, MW, SD, SZ, UG, ZW, AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG

AU 9890705	A1	19990222	AU 1998-90705	19980727
EP 1005553	A1	20000607	EP 1998-942648	19980727

R: AT, BE, CH, DE, DK, ES, FR, GB, IT, LI, NL, SE, IE

PRAI DE 1997-19732829 19970730
WO 1998-EP4723 19980727

AB The invention concerns the prodn. of recombinant S-layer protein expressed in Gram-neg. prokaryote or eukaryote host cells using the sbsA and sbsB clones of the Bacillus stearothermophilus PV72, that code for the S-layer protein and the prokaryote signal peptide; the vector also contains inserts at convenient sites that code for various peptides, e.g. cysteine residues, DNA-binding epitopes, metal-binding epitopes, allergens, antigens, streptavidin, enzymes etc. In case the **fusion protein** is expressed in eukaryotes, the vector includes sequences coding for eukaryote signal peptides. The host cell contains at least two types of genes that code for the a non-modified S-layer protein and for a modified S-layer protein that is fused with a peptide used biochem. reactions. E.coli is a typical host cell.

L4 ANSWER 2 OF 12 EMBASE COPYRIGHT 2000 ELSEVIER SCI. B.V.DUPLICATE 1
AN 1999302348 EMBASE
TI Bacterial ghosts as drug carrier and targeting vehicles.
AU Huter V.; Szostak M.P.; Gampfer J.; Prethaler S.; Wanner G.; Gabor F.; **Lubitz W.**
CS V. Huter, Inst. Microbiology and Genetics, University of Vienna, Dr. Bohrgasse 9, A-1030 Vienna, Austria
SO Journal of Controlled Release, (1999) 61/1-2 (51-63).
Refs: 41
ISSN: 0168-3659 CODEN: JCREEC
PUI S 0168-3659(99)00099-1
CY Netherlands
DT Journal; Article
FS 029 Clinical Biochemistry
030 Pharmacology
037 Drug Literature Index
039 Pharmacy
LA English
SL English
AB A novel system for the packaging of drugs as well as vaccines is presented. Bacterial ghosts are intact, non-denatured bacterial envelopes that are created by lysis of bacteria through the expression of cloned phage PhiX174 gene E. Inhibition of induced E-mediated lysis by MgSO4, harvesting of cells by centrifugation, and resuspension in low-ionic-strength buffers leads to rapid, violent lysis and results in empty bacterial envelopes with large (approximately 1 .mu.m in diameter) openings. The construction of plasmid pAV1, which encodes a streptavidin

fusion protein with an N-terminal membrane anchor sequence, allows the loading of the inner side of the cytoplasmic membrane with streptavidin. The functionality and efficacy of binding of even large biotinylated compounds in such streptavidin ghosts (SA-ghosts) was assessed using the enzyme alkaline phosphatase. The successful binding of biotinylated fluorescent dextran, as well as fluorescent DNA complexed with biotinylated polylysine, was demonstrated microscopically. The display by bacterial ghosts of morphological and antigenic surface structures of their living counterparts permits their attachment to target tissues such as the mucosal surfaces of the gastrointestinal and respiratory tract, and their uptake by phagocytes and M cells. In consequence, SA-ghosts are proposed as drug carriers for site-specific drug delivery. Copyright (C) 1999 Elsevier Science B.V.

L4 ANSWER 3 OF 12 EMBASE COPYRIGHT 2000 ELSEVIER SCI. B.V.DUPLICATE 2
AN 97348874 EMBASE
DN 1997348874
TI Proline 21, a residue within the .alpha.-helical domain of .PHI.X174 lysis protein E, is required for its function in Escherichia coli.
AU Witte A.; Schrot G.; Schon P.; **Lubitz W.**
CS A. Witte, Institute Microbiology and Genetics, University of Vienna, Dr Bohrgasse 9, A-1030 Vienna, Austria
SO Molecular Microbiology, (1997) 26/2 (337-346).
Refs: 50
ISSN: 0950-382X CODEN: MOMIEE
CY United Kingdom
DT Journal; Article
FS 004 Microbiology
LA English
SL English
AB .PHI.X174 lysis protein E-mediated lysis of Escherichia coli is characterized by a protein E-specific fusion of the inner and outer membrane and formation of a transmembrane tunnel structure. In order to understand the fusion process, the topology of protein E within the envelope complex of E. coli was investigated. Proteinase K protection studies showed that, during the time course of protein E-mediated lysis process, more of the **fusion protein** E-FXa-streptavidin gradually became accessible to the protease at the cell surface. These observations postulate a conformational change in protein E during induction of the lysis process by movement of the C-terminal end of the protein throughout the envelope complex from the inner side to the outer side spanning the entire pore and fusing the inner and outer membranes at distinct areas. The initiation mechanism for such a conformational change could be the cis-trans isomerization of proline residues within .alpha.-helical membrane-spanning segments. Conversion of proline 21, presumed to be in the membrane-embedded .alpha.-helix of protein E, to alanine, glycine, serine and valine, respectively, resulted in lysis-negative E mutant proteins. Proteinase K accessibility studies using streptavidin as a reporter fused to the P21G mutant protein showed that the C-terminal part of the **fusion protein** is not translocated to the outer side of the membrane, suggesting that this proline residue is essential for the correct folding of protein E within the cell wall complex of E. coli. Oligomerization of protein P21G-StrpA was not disturbed.

L4 ANSWER 4 OF 12 USPATFULL
AN 95:105569 USPATFULL
TI Immunogens comprising the non-lytic membrane spanning domain of bacteriophages MS2 or PhiX174
IN **Lubitz, Werner**, Munich, Germany, Federal Republic of

Szostak, Michael P., Munich, Germany, Federal Republic of
PA Boehringer Mannheim GmbH, Mannheim, Germany, Federal Republic of (non-U.S. corporation)
PI US 5470573 19951128
WO 9113155 19910905
AI US 1992-924028 19920930 (7)
WO 1991-EP308 19910219
19920930 PCT 371 date
19920930 PCT 102(e) date
DT Utility
EXNAM Primary Examiner: Nucker, Christine M.; Assistant Examiner: Tuscan, Michael
LREP Felfe & Lynch
CLMN Number of Claims: 8
ECL Exemplary Claim: 1
DRWN 5 Drawing Figure(s); 2 Drawing Page(s)
LN.CNT 961
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
AB The invention concerns a carrier-bound recombinant protein obtainable by expression of a **fusion protein** gene in gram-negative bacteria which codes for at least one hydrophobic non-lytically active protein domain capable of membrane integration as well as the recombinant protein and of a gene which codes for a lytically active membrane protein from bacteriophages or a lytically active toxin release gene or lytically active partial sequences thereof and isolation of the carrier-bound recombinant protein from the culture broth. The recombinant protein is thereby firmly incorporated into the cell wall complex of gram-negative bacteria via a target sequence. Furthermore the invention concerns a recombinant DNA for the production of the protein, the production process as well as the use of carrier-bound recombinant proteins according to the present invention for immunization and as vaccines.

L4 ANSWER 5 OF 12 EMBASE COPYRIGHT 2000 ELSEVIER SCI. B.V.DUPLICATE 3
AN 95254950 EMBASE
DN 1995254950
TI Two-stage model for integration of the lysis protein E of .PHI.X174 into the cell envelope of Escherichia coli.
AU Schon P.; Schrot G.; Wanner G.; **Lubitz W.**; Witte A.
CS Institute Microbiology and Genetics, University of Vienna, Dr. Bohr Gasse 9,A-1030 Vienna, Austria
SO FEMS Microbiology Reviews, (1995) 17/1-2 (207-212).
ISSN: 0168-6445 CODEN: FMREE4
CY Netherlands
DT Journal; Conference Article
FS 004 Microbiology
LA English
SL English
AB As a tool for determining the topology of the small, 91-amino acid .PHI.X174 lysis protein:E within the envelope complex of Escherichia coli, a lysis active fusion of protein E with streptavidin (E-FXa-StrpA) was used. The E-FXa-StrpA **fusion protein** was visualised using immune electron microscopy with gold-conjugated anti-streptavidin antibodies within the envelope complex in different orientations. At the distinct areas of lysis characteristic for protein E, the C-terminal end of the **fusion protein** was detected at the surface of the outer membrane, whereas at other areas the C-terminal portion of the protein was located at the cytoplasmic side of the inner membrane. These results suggest that a conformational change of protein E is necessary to induce the lysis process, an assumption supported by proteinase K protection studies. The immune electron microscopic data and the

proteinase K accessibility studies of the E-FXa-StrA **fusion protein** were used for the working model of the E-mediated lysis divided into three phases: phase I is characterised by integration of protein E into the inner membrane without a cytoplasmic status in a conformation with its C-terminal part facing the cytoplasmic side; phase 2 is characterised by a conformational change of the protein transferring the C-terminus across the inner membrane; phase 3 is characterised by a fusion of the inner and outer membranes and is associated with a transfer of the C-terminal domain of protein E towards the surface of the outer membrane of E. coli.

L4 ANSWER 6 OF 12 EMBASE COPYRIGHT 2000 ELSEVIER SCI. B.V.DUPLICATE 4
AN 94289533 EMBASE
DN 1994289533
TI Production of Vibrio cholerae ghosts (VCG) by expression of a cloned phage lysis gene: Potential for vaccine development.
AU Eko F.O.; Szostak M.P.; Wanner G.; **Lubitz W.**
CS Inst. of Microbiology and Genetics, University of Vienna, Biocenter, Dr Bohrgasse 9,1030 Vienna, Austria
SO Vaccine, (1994) 12/13 (1231-1237).
ISSN: 0264-410X CODEN: VACCDE
CY United Kingdom
DT Journal; Article
FS 004 Microbiology
026 Immunology, Serology and Transplantation
037 Drug Literature Index
LA English
SL English
AB The protein E-specific lysis mechanism of the Escherichia coli-specific bacteriophage PhiX174 was employed to produce Vibrio cholerae ghosts (VCG). VCG consist of both rounded and collapsed cells that have lost their cytoplasmic contents through an E-specific hole in the cell envelope. These ghosts are proposed as non-living material for immunization against cholera. A specific membranes anchor sequence was used to insert the human immunodeficiency virus type 1 (HIV-1) reverse transcriptase (RT) **fusion protein** into the cell envelope of V. cholerae. The identity of the expression products was confirmed by Western blot analysis employing an RT-specific monoclonal antibody. HIV-1 RT was chosen as a model for the purpose of evaluating heterologous gene expression in V. cholerae and the carrier potential of VCG. Intraperitoneal immunization of mice was used to evaluate the immunogenic potential of VCG. Preliminary results showed significant seroconuersions to intact whole-cell vibrio antigens in mice immunized with VCG or a heat-killed whole-cell vibrio preparation.

L4 ANSWER 7 OF 12 USPATFULL
AN 91:104101 USPATFULL
TI Recombinant DNA, process for the production thereof and the use thereof
IN **Lubitz, Werner**, Munich, Germany, Federal Republic of
Harkness, Robin E., Tubingen, Germany, Federal Republic of
PA Boehringer Mannheim GmbH, Mannheim, Germany, Federal Republic of (non-U.S. corporation)
PI US 5075223 19911224
AI US 1988-191531 19880509 (7)
PRAI DE 1987-3715840 19870512
DT Utility
EXNAM Primary Examiner: Martinell, James
LREP Felfe & Lynch
CLMN Number of Claims: 21
ECL Exemplary Claim: 1
DRWN 4 Drawing Figure(s); 3 Drawing Page(s)
LN.CNT 320
CAS INDEXING IS AVAILABLE FOR THIS PATENT.

AB The present invention provides a recombinant DNA, wherein it contains DNA sequences coding for the N-terminal membrane-penetrating domain of the E-protein of the phage .phi.X 174 and DNA sequences coding for the C-terminal membrane-penetrating domain of the L-protein of the phage MS2
and the DNA sequences of both phages are connected by a DNA sequence coding for a hydrophilic flexible amino acid sequence.

The present invention also provides a process for the production of this
recombinant DNA.

Furthermore, the present invention provides for the use of the recombinant DNA and of a plasmid containing it for obtaining eukaryotic and prokaryotic metabolic products and gene-technologically produced proteins.

L4 ANSWER 8 OF 12 CAPLUS COPYRIGHT 2000 ACS
AN 1992:1761 CAPLUS
DN 116:1761
TI Membrane-anchoring of heterologous proteins in recombinant hosts for use as antigens
IN **Lubitz, Werner;** Szostak, Michael P.
PA Boehringer Mannheim G.m.b.H., Germany
SO PCT Int. Appl., 46 pp.
CODEN: PIXXD2
DT Patent
LA German
FAN.CNT 1

	PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
PI	WO 9113155	A1	19910905	WO 1991-EP308	19910219
	W: AU, FI, HU, JP, SU, US				
	RW: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LU, NL, SE				
	DE 4005874	A1	19911107	DE 1990-4005874	19900224
	AU 9172373	A1	19910918	AU 1991-72373	19910219
	EP 516655	A1	19921209	EP 1991-903789	19910219
	EP 516655	B1	19940504		
	R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, SE				
	JP 05503014	T2	19930527	JP 1991-503980	19910219
	AT 105335	E	19940515	AT 1991-903789	19910219
	US 5470573	A	19951128	US 1992-924028	19920930
PRAI	DE 1990-4005874	19900224			
	EP 1991-903789	19910219			
	WO 1991-EP308	19910219			

AB Antigenic proteins are prepd. with a Gram-neg. bacteria contg. a gene encoding a lytic protein by expression of a chimeric gene for a **fusion protein** of a membrane-anchoring domain and the antigen. Plasmid pAV5 encoding a streptavidin-phage MS2 protein L **fusion protein** and a plasmid contg. the protein E gene of phage .phi.X174 under control of the temp. sensitive .lambda. repressor-.lambda. promoter/operator system were prepd. Escherichia coli was transformed with these plasmids, cultured to permit cell growth and **fusion protein** synthesis, then temp.-shifted to cause protein E prodn. and cell lysis. The bacterial ghosts prepd. were incubated with a hepatitis B core antigen-biotin conjugate to prep. an immunogen.

L4 ANSWER 9 OF 12 BIOSIS COPYRIGHT 2000 BIOSIS
AN 1991:422040 BIOSIS
DN BR41:71585
TI RECOMBINANT BACTERIAL GHOSTS AS MULTIVACCINE VEHICLES.
AU SZOSTAK M; **LUBITZ W**
CS INST. MICROBIOL. GENETICS, UNIV. VIENNA, A-1090 VIENNA, AUSTRIA.
SO CHANOCK, R. M., ET AL. (ED.). VACCINES (COLD SPRING HARBOR), VOL. 91.

MODERN APPROACHES TO NEW VACCINES INCLUDING PREVENTION OF AIDS; EIGHTH ANNUAL MEETING, COLD SPRING HARBOR, NEW YORK, USA, SEPTEMBER 1990. XXIII+441P. COLD SPRING HARBOR LABORATORY PRESS: COLD SPRING HARBOR, NEW YORK, USA. ILLUS. PAPER. (1991) 0 (0), 409-414.
CODEN: VMAVEA. ISBN: 0-87969-367-3.
DT Conference
FS BR; OLD
LA English

L4 ANSWER 10 OF 12 LIFESCI COPYRIGHT 2000 CSA
AN 91:81531 LIFESCI
TI Recombinant DNA, process for the production thereof and the use thereof.
AU **Lubitz**, W.; Harkness, R.E.
CS Boehringer Mannheim GmbH, Mannheim (FRG)
PI US 5075223 1991
SO (1991) . US Cl. 435/69.1; Int. Cl. C12N 15/00, 15/11, C12P 21/00..
DT Patent
FS W
LA English
AB Recombinant DNA sequence comprising: a DNA sequence coding for the non-lytic N-terminal membrane penetrating domain of the E protein of phage phi X174, a DNA sequence coding for a hydrophobic, flexible amino acid sequence, and a DNA sequence coding for the non-lytic, C-terminal membrane penetrating domain of the L protein of phage MS2, wherein it is positioned in between and links the first 2 sequences, and wherein said recombinant DNA sequence codes for a lytic **fusion protein**.

L4 ANSWER 11 OF 12 BIOTECHDS COPYRIGHT 2000 DERWENT INFORMATION LTD
AN 1991-02593 BIOTECHDS
TI Recombinant bacterial ghosts as vaccines; phage phi-X174 and phage MS2 L protein **fusion protein** gene cloning and expression in Escherichia coli ghost; plasmid pKSEL5 or plasmid pMTV1 recombinant vaccine vector (conference paper)
AU Szostak M; Wanner G; **Lubitz W**
LO Institute of Microbiology and Genetics, University of Vienna, Althanstrasse 14, 1090 Vienna, Austria.
SO Res.Microbiol.; (1990) 141, 7-8, 1005-07
CODEN: RMCREW
DT Journal
LA English
AB The potential of Gram-negative bacterial ghosts (e.g. Escherichia coli empty cell envelopes) carrying recombinant viral proteins as novel immunogens was explored. The membrane target system for insertion of viral proteins was based on the hydrophobic membrane spanning domains of truncated E and L proteins of phage phi-X174 and phage MS2, respectively. Foreign proteins were inserted via N-terminus fusion with E, C-terminus fusion with L or internal fusion with N- and C-termini of target sequences. A new chimeric E-L gene was constructed by combining the 5' and 3' sequences of E and L genes, respectively, and was used for construction of new vectors. Plasmid pKSEL5 and plasmid pMTV1 contained DNA cassettes with antibiotic-resistance selectable marker genes, membrane targeting sequences with the E-L sequence under control of the lac system, and a functional phi-X174 lysis gene E, under control of phage lambda pL or pR and cI857 sequences. Using this system, immunogens could be inserted into a highly immunostimulatory environment, with no size limitation, for production of inexpensive and safe recombinant vaccines. (3 ref)

L4 ANSWER 12 OF 12 CAPLUS COPYRIGHT 2000 ACS
AN 1989:226612 CAPLUS
DN 110:226612
TI Use and manufacture of metabolites and recombinant proteins
IN **Lubitz, Werner**; Harkness, Robin Edmond
PA Boehringer Mannheim G.m.b.H., Fed. Rep. Ger.
SO Eur. Pat. Appl., 8 pp.
CODEN: EPXXDW
DT Patent
LA German
FAN.CNT 1

	PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
PI	EP 291021	A2	19881117	EP 1988-107519	19880510
	EP 291021	A3	19900613		
	EP 291021	B1	19930210		
	R: AT, BE, CH, DE, ES, FR, GB, GR, IT, LI, LU, NL, SE				
	DE 3715840	A1	19881201	DE 1987-3715840	19870512
	US 5075223	A	19911224	US 1988-191531	19880509
	AT 85648	E	19930215	AT 1988-107519	19880510
	JP 63287489	A2	19881124	JP 1988-113707	19880512
	JP 07102136	B4	19951108		
PRAI	DE 1987-3715840	19870512			
	EP 1988-107519	19880510			

AB A chimeric gene encoding the N-terminal membrane-spanning domain of the E protein of OX174 fused to the C-terminal membrane-spanning domain of the L protein of MS2 is constructed. Expression of this gene in eukaryotic or prokaryotic cells, or in recombinant cells, results in partial or complete lysis of the cell membrane, which facilitates isolation of the desired product (metabolite, recombinant protein, etc.). Thus, plasmid pRM17, contg. the described chimeric gene under control of the .lambda.P1 promoter, was constructed. Escherichia coli were transformed with the plasmid and cultured at 28.degree.. Upon raising the temp. to 42.degree. expression of the chimeric gene was induced and the microbes were lysed.

=> s l1 and s layer protein

L5 36 L1 AND S LAYER PROTEIN

=> dup rem l5

PROCESSING COMPLETED FOR L5
L6 14 DUP REM L5 (22 DUPLICATES REMOVED)

=> d bib ab 1-14

L6 ANSWER 1 OF 14 EMBASE COPYRIGHT 2000 ELSEVIER SCI. B.V. DUPLICATE 1
AN 2000287774 EMBASE
TI The transposable element IS4712 prevents S-layer gene (sbsA) expression in Bacillus stearothermophilus and also affects the synthesis of altered surface layer proteins.
AU Scholz H.; Hummel S.; Witte A.; **Lubitz W.**; Kuen B.
CS H. Scholz, Institute of Animal Hygiene, Public Veterinary Health, An den Tierkliniken 43, 04103 Leipzig, Germany. scholz@vetmed.uni-leipzig.de
SO Archives of Microbiology, (2000) 174/1-2 (97-103).
Refs: 19
ISSN: 0302-8933 CODEN: AMICCW
PUI S002030000181
CY Germany
DT Journal; Article

FS 004 Microbiology
029 Clinical Biochemistry
LA English
SL English
AB Cell surface (S)-**layer protein** synthesis in Bacillus stearothermophilus PV72/p6 is blocked when cells are grown at elevated temperature. From a culture exhibiting the S-layer-negative phenotype, the S-layer deficient mutant T5 (SbsA-) was isolated. Genetic analysis of the S-layer-encoding gene (sbsA) of mutant T5 revealed an insertion element (IS4712) integrated into the upstream regulatory region of the S-layer gene, thereby blocking sbsA transcription. The insertion element consists of 1371 base pairs which are flanked by two perfect inverted terminal repeats. Sequence similarity to other transposases of the IS4 family was detected. DNA-DNA hybridizations demonstrated that multiple homologues of IS4712 were also present within the genomes of several other thermophilic bacillus isolates. Attempts to isolate SbsA+ revertants failed. Instead, cells with altered surface proteins were detected. The synthesis of the altered S-layer proteins was correlated with the presence of IS4712 along with the occurrence of deletions in the sbsA coding region. Furthermore imprecise excision of IS4712 was detected.

This work demonstrated that B. stearothermophilus is able to express at least four different S-layer proteins and that blocking of sbsA transcription by the insertion element IS4712 is associated with the expression of altered surface proteins.

L6 ANSWER 2 OF 14 BIOTECHDS COPYRIGHT 2000 DERWENT INFORMATION LTD
AN 1999-04719 BIOTECHDS
TI Producing S-layer proteins in Gram-negative bacteria or eukaryotes; for use as recombinant vaccine
AU **Lubitz** W
PA Lubitz W
LO Vienna, Austria.
PI DE 19732829 4 Feb 1999
AI DE 1997-1032829 30 Jul 1997
PRAI DE 1997-1032829 30 Jul 1997
DT Patent
LA German
OS WPI: 1999-122189 [11]
AB A means of producing **S-layer protein** (I) is claimed. It involves transforming a Gram-negative prokaryotic cell with a nucleic acid that encodes (I) linked to a signal peptide that encodes a protein which causes integration of (I) into the external or cytoplasmic membrane, or secretion of (I) into the periplasmic space or extracellular medium. The bacterium is then cultured, and (I) recovered from the membrane, periplasmic space, or medium. Alternatively a eukaryotic cell can be used as the host, in which case the signal peptide promotes integration of (I) into the cytoplasmic membrane, or an organelle, or induces secretion of (I) into the extracellular medium. Also claimed is a nucleic acid (II) that encodes (I) and the signal peptide, optionally including heterologous peptide inserts. The claims also cover a vector containing (II), and Gram-positive prokaryotic or eukaryotic cells transformed by that vector (e.g. plasmid pMAL-A used to transform Escherichia coli DH5-alpha. (I) are useful as vaccines, reactors, and universal carrier molecules. (33pp)

L6 ANSWER 3 OF 14 BIOTECHDS COPYRIGHT 2000 DERWENT INFORMATION LTD
AN 1999-11466 BIOTECHDS
TI Extended recombinant bacterial ghost system; ghost cell production and foreign gene and antigen expression for use as a recombinant combination vaccine (conference paper)
AU **Lubitz** W; Witte A; Eko F O; Kamal M; Jechlinger W; Brand E; Marchart J; Haidinger W; Huter V; Felnerova D; Stralis-Alves N;

Lechleitner S; Melzer H; Szostak M P; Resch S; Nader H; Kuen B; Mayr B; Mayrhofer P; Geretschlager R; Haslberger A; Hensel A

CS Univ.Vienna-Inst.Microbiol.Genet.; EVAX-Technol.; Univ.Leipzig-Inst.Anim.Hyg.Vet.Public-Health

LO Institute of Microbiology and Genetics, University of Vienna, Dr. Bohrgasse 9, A-1030 Vienna, Austria.
Email: oldfox@gem.univie.ac.at

SO J.Biotechnol.; (1999) 73, 2-3, 261-73
CODEN: JBITD4 ISSN: 0168-1656
New Approaches in Vaccine Development 1997, Australian Society of Biotechnology, Vienna, Austria, 1997.

DT Journal

LA English

AB Controlled expression of cloned PhiX174 gene E in Gram-negative bacteria results in lysis of the bacteria by formation of an E-specific transmembrane tunnel structure built through the cell envelope complex. These bacterial ghosts from a variety of bacteria were used as non-living candidate vaccines. In a recombinant ghost system, the desired foreign proteins are attached to the inside of the inner membrane as fusions with specific anchor sequences. Because the ghosts have a sealed periplasmic and the proteins can be exported into this space the capacity of the ghost or recombinant ghost systems can be vastly extended, therefore making them capable carriers of foreign antigens. The recombinant ghosts can also express **S-layer protein** (shell-like structure), which can carry foreign gene epitopes, which further extends the possibilities of ghost carriers. The ghost also have inherent adjuvant properties, so they can be used as adjuvants in combination with subunit vaccines. There is no limitations on the size of foreign antigens which can be inserted into the ghosts and so they may be used as adjuvant free combination vaccines. (32 ref)

L6 ANSWER 4 OF 14 EMBASE COPYRIGHT 2000 ELSEVIER SCI. B.V.DUPLICATE 3

AN 1999088729 EMBASE

TI Self-assembly product formation of the Bacillus stearothermophilus PV72/p6 **S-layer protein** SbsA in the course of autolysis of Bacillus subtilis.

AU Howorka S.; Sara M.; **Lubitz W.**; Kuen B.

CS B. Kuen, Institut Mikrobiologie and Genetik, Universitat Wien, Dr. Bohrgasse 9, A-1030 Vienna, Austria. oetzi@gem.univie.ac.at

SO FEMS Microbiology Letters, (1999) 172/2 (187-196).
Refs: 20
ISSN: 0378-1097 CODEN: FMLED7

PUI S 0378-1097(99)00040-3

CY Netherlands

DT Journal; Article

FS 004 Microbiology

LA English

SL English

AB In order to achieve high level expression and to study the release of a protein capable of self-assembly, the gene encoding the crystalline cell surface (**S-layer**) **protein** SbsA of Bacillus stearothermophilus PV72/p6, including its signal sequence, was cloned and expressed in Bacillus subtilis. To obtain high level expression, a tightly regulated, xylose-inducible, stably replicating multicopy-plasmid vector was constructed. After induction of expression, the S-**layer protein** made up about 15% of the total cellular protein content, which was comparable to the SbsA content of B. stearothermophilus PV72/p6 cells. During all growth stages, SbsA was poorly secreted to the ambient cellular environment by B. subtilis.

Extraction of whole cells with guanidine hydrochloride showed that in late stationary growth phase cells 65% of the synthesised SbsA was retained in the peptidoglycan-containing layer, indicating that the rigid cell wall layer was a barrier for efficient SbsA secretion. Electron microscopic investigation revealed that SbsA release from the peptidoglycan-containing layer started in the late stationary growth phase at distinct sites at the cell surface leading to the formation of extracellular self-assembly products which did not adhere to the cell wall surface. In addition, intracellular sheet-like SbsA self-assembly products which followed the curvature of the cell became visible in partly lysed cells. Intracellularly formed self-assembly products remained intact even after complete lysis of the rigid cell envelope layer. Copyright (C) 1999 Federation of European Microbiological Societies.

L6 ANSWER 5 OF 14 BIOTECHDS COPYRIGHT 2000 DERWENT INFORMATION LTD
AN 1997-11103 BIOTECHDS
TI Preparation of S-layer proteins by expressing sbs-A gene in Gram-negative bacterium;
for use as e.g. vaccine or adjuvant
AU **Lubitz W**; Sleytr U; Kuen B
PA Lubitz W; Sleytr U
LO Vienna, Austria.
PI DE 19603649 7 Aug 1997
AI DE 1996-1003649 1 Feb 1996
PRAI DE 1996-1003649 1 Feb 1996
DT Patent
LA German
OS WPI: 1997-394558 [37]
AB A new method for the preparation of **S-layer protein** (I) involves transforming a Gram-negative prokaryote, preferably Escherichia coli, with a nucleic acid encoding (I) contained on a vector, and culturing the transformed cells. The nucleic acid may contain one or more inserts, preferably encoding Cys residues, regions with many charged amino acids or Tyr, DNA-or metal-binding epitopes, immune, allergenic or antigenic epitopes, streptavidin, enzymes or cytokine- or antibody-binding proteins. (I) is useful as a recombinant vaccine or adjuvant, especially when combined with a bacterial ghost that may contain additional epitopes in its membrane. Other uses, depending on the inserted protein, include (a) universal adjuvant for biotinylated reactants for immunological or hybridization assays, (b) induction of immune responses, (c) reagent for removing cytokine or toxin from serum, (d) molecular spinning nozzle and (e) molecular laser. When expressed in Gram-negative cells, (I) is produced in the form of monomolecular layers rather than as inclusion bodies as in Gram-positive bacteria. (31pp)

L6 ANSWER 6 OF 14 CAPLUS COPYRIGHT 2000 ACS
AN 1997:536912 CAPLUS
DN 127:201021
TI Expression of S-layer proteins in Gram-negative bacteria and recombinant chimeric S-layer proteins for use as vaccines
IN **Lubitz, Werner**; Sleytr, Uwe; Kuen, Beatrix; Truppe, Michaela; Howorka, Stefan; Resch, Stepanka; Schroll, Gerhard; Sara, Margit
PA Lubitz, Werner, Austria; Sleytr, Uwe; Kuen, Beatrix; Truppe, Michaela; Howorka, Stefan; Resch, Stepanka; Schroll, Gerhard; Sara, Margit
SO PCT Int. Appl., 65 pp.
CODEN: PIXXD2
DT Patent
LA German
FAN.CNT 1

	PATENT NO.	KIND	OATE	APPLICATION NO.	DATE
PI	WO 9728263	A1	19970807	WO 1997-EP432	19970131
	W: AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GE, HU, IL, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MO, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, TJ, TM, TR, TT, UA, UG, US, UZ, VN, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM				
	RW: KE, LS, MW, SD, SZ, UG, AT, BE, CH, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, BF, BJ, CF, CG, CI, CM, GA, GN, ML, MR, NE, SN, TD, TG				
	OE 19603649	A1	19970807	DE 1996-19603649	19960201
	CA 2245584	AA	19970807	CA 1997-2245584	19970131
	AU 9717203	A1	19970822	AU 1997-17203	19970131
	AU 713999	B2	19991216		
	EP 882129	A1	19981209	EP 1997-904360	19970131
	R: AT, BE, CH, DE, OK, ES, FR, GB, GR, IT, LI, LU, NL, SE, MC, PT, IE, FI				
	CN 1213402	A	19990407	CN 1997-192940	19970131
	JP 2000503850	T2	20000404	JP 1997-527307	19970131
PRAI	OE 1996-19603649	19960201			
	WO 1997-EP432	19970131			

AB The invention concerns processess for the recombinant prepn. of S-layer proteins in Gram-neg. host cells. In addn., the nucleotide sequence of a new S-layer gene, the sbsB gene of Bacillus stearothermophilus, and a process for prepn. of modified S-layer proteins is disclosed.

Recombinant

Escherichia coli expressing the sbsA gene of B. stearothermophilus and chimeric sbsA genes encoding SbsA into which various peptides, proteins and enzymes have been inserted were prepd. and cultured to produce the proteins.

L6 ANSWER 7 OF 14 EMBASE COPYRIGHT 2000 ELSEVIER SCI. B.V.DUPLICATE 4

AN 97071257 EMBASE

DN 1997071257

TI Molecular characterization of the Bacillus stearothermophilus PV72 S-layer gene sbsB induced by oxidative stress.

AU Kuen B.; Koch A.; Asenbauer E.; Sara M.; **Lubitz W.**

CS B. Kuen, Inst. of Microbiology and Genetics, Biocenter Vienna, Dr. Bohrg. 9, 1030 Vienna, Austria. oetzi@gem.univie.ac.at

SO Journal of Bacteriology, (1997) 179/5 (1664-1670).
Refs: 42
ISSN: 0021-9193 CODEN: JOBAAY

CY United States

OT Journal; Article

FS 004 Microbiology

LA English

SL English

AB **S-layer protein** variation from a hexagonally ordered (SbsA; 130 kDa) to a obliquely ordered (SbsB; 98 kDa) protein in Bacillus stearothermophilus PV72 is mediated by an increased oxygen supply. To elucidate the molecular basis of **S-layer protein** variation in B. stearothermophilus PV72, the sbsB gene, ceding for the 98-kDa protein, was cloned by means of inverse PCR technology and sequenced. The sbsB coding region cloned in pUC18 was expressed in Escherichia coli, without its own regulatory upstream sequences but with its putative transcriptional terminator. The reading frame of sbsB (2,760 nucleotides) is predicted to encode a protein of 920 amino acids, including the signal sequence. Amino acid sequence comparison of SbsA and SbsB did not reveal any significant homology. The expression of sbsB in E. coli resulted in an accumulation of SbsB self-assembly products in the cytoplasm.

L6 ANSWER 8 OF 14 EMBASE COPYRIGHT 2000 ELSEVIER SCI. B.V.DUPLICATE 5

AN 97267948 EMBASE
DN 1997267948
TI IV. Molecular biology of S-layers.
AU Bahl H.; Scholz H.; Bayan N.; Chami M.; Leblon G.; Gulik-Krzywicki T.; Shechter E.; Fouet A.; Mesnage S.; Tosi-Couture E.; Gounon P.; Mock M.; De Macario E.C.; Macario A.J.L.; Fernandez-Herrero L.A.; Olabarria G.; Berenguer J.; Blaser M.J.; Kuen B.; **Lubitz W.**; Sara M.; Pouwels P.H.; Kolen C.P.A.M.; Boot H.J.; Palva A.; Truppe M.; Howorka S.; Schroll G.; Lechleitner S.; Resch S.
CS Dr. N. Bayan, Laboratoire des Biomembranes, URA 1116 CNRS, Universite de Paris-Sud, F-91405 Orsay, France
SO FEMS Microbiology Reviews, (1997) 20/1-2 (47-98).
Refs: 197
ISSN: 0168-6445 CODEN: FMREE4
PUI S 0168-6445(97)00050-8
CY Netherlands
DT Journal; General Review
FS 004 Microbiology
LA English
SL English
AB In this chapter we report on the molecular biology of crystalline surface layers of different bacterial groups. The limited information indicates that there are many variations on a common theme. Sequence variety, antigenic diversity, gene expression, rearrangements, influence of environmental factors and applied aspects are addressed. There is considerable variety in the S-layer composition, which was elucidated by sequence analysis of the corresponding genes. In Corynebacterium glutamicum one major cell wall protein is responsible for the formation of a highly ordered, hexagonal array. In contrast, two abundant surface proteins form the S-layer of Bacillus anthracis. Each protein possesses three S-layer homology motifs and one protein could be a virulence factor.
The antigenic diversity and ABC transporters are important features, which have been studied in methanogenic archaea. The expression of the S-layer components is controlled by three genes in the case of Thermus thermophilus. One has repressor activity on the S-layer gene promoter, the second codes for the **S-layer protein**. The rearrangement by reciprocal recombination was investigated in Campylobacter fetus. 7-8 S-layer proteins with a high degree of homology at the 5' and 3' ends were found. Environmental changes influence the surface properties of Bacillus stearothermophilus. Depending on oxygen supply, this species produces different S-layer proteins. Finaly, the molecular bases for some applications are discussed. Recombinant S-layer fusion proteins have been designed for biotechnology.

L6 ANSWER 9 OF 14 BIOTECHDS COPYRIGHT 2000 DERWENT INFORMATION LTD
AN 1996-05577 BIOTECHDS
TI Nucleic acid encoding signal peptide of Bacillus stearothermophilus **S-layer protein**; for secretion of protein which has a high lysine content
AU **Lubitz W**
PA Vogelbusch
PI DE 4425527 25 Jan 1996
AI DE 1994-4425527 19 Jul 1994
PRAI DE 1994-4425527 19 Jul 1994
DT Patent
LA German
OS WPI: 1996-077933 [09]
AB A nucleic acid (I) encoding a functional signal peptide (SP) is new which is selected from: (a) the SP-encoding portion of a 3,706 bp sequence, (b)

a sequence corresponding to (a) taking into account the degeneracy of the genetic code or (c) a sequence with at least 90% homology to (a) or (b). Also claimed are: (1) (I) operatively linked at its 3'-terminus to a protein-encoding nucleic acid, (2) (I) or the nucleic acid of (1) operatively linked at its 5'-terminus to an expression control sequence, (3) a protein encoded by a nucleic acid, (4) a recombinant vector containing at least 1 copy of a nucleic acid, (5) a host cell transformed with a nucleic acid or vector and (6) an expression control sequence. This process is useful for the production of Bacillus stearothermophlis **S-layer protein**, which has a lysine content of at least 10%. Optimally the protein in hydrolyzed and the amino acids recovered. (11pp)

L6 ANSWER 10 OF 14 EMBASE COPYRIGHT 2000 ELSEVIER SCI. B.V.DUPLICATE 7
AN 96051831 EMBASE
DN 1996051831
TI Heterologous expression and self-assembly of the **S-layer protein** SbsA of Bacillus stearothermophilus in Escherichia coli.
AU Kuen B.; Sara M.; **Lubitz W.**
CS Institut fur Mikrobiologie/Genetik, Universitat Wien, Dr. Bohrg 9,A-1030 Wien, Austria
SO Molecular Microbiology, (1996) 19/3 (495-503).
ISSN: 0950-382X CODEN: MOMIEE
CY United Kingdom
DT Journal; Article
FS 004 Microbiology
LA English
SL English
AB The cell surface of Bacillus stearothermophilus PV72 is covered by a regular surface layer (S-layer) composed of a single species of protein, SbsA, with a molecular weight of 130000. Recently, the sequence of the corresponding gene (sbsA) has been determined. The SbsA coding region including the signal sequence was cloned as a polymerase chain reaction (PCR) product into a low-copy-number vector under the transcriptional control of the .lambda.pL promoter. Expression of sbsA was shown to be thermally inducible from the resulting vector pBK4 in a strain of Escherichia coli expressing the .lambda.cI857 from the chromosome. As shown by ultrathin sectioning of whole cells and immunogold labelling using SbsA-specific antibodies, expression of sbsA in E. coli led to accumulation of sheet-like self-assembling products of the protein in the cytoplasm. No SbsA protein was detected either in the periplasm or in the supernatant fractions. Long-term expression of sbsA from pBK4, including in the late stationary phase, did not lead to degradation of SbsA.

L6 ANSWER 11 OF 14 CAPLUS COPYRIGHT 2000 ACS
AN 1996:492078 CAPLUS
DN 125:213353
TI Analysis of S-layer proteins and genes
AU Kuen, Beatrix; **Lubitz, Werner**
CS Austria
SO Cryst. Bact. Cell Surf. Proteins (1996), 77-102. Editor(s): Sleytr, Uwe B. Publisher: Landes, Austin, Tex.
CODEN: 63EDAO
DT Conference; General Review
LA English
AB A review with 75 refs. Surface layers (S-layers) are regularly ordered proteins found as the outermost cell envelope component of many bacteria. The authors discuss similarities and common characteristics of currently know S-layer genes.

L6 ANSWER 12 OF 14 LIFESCI COPYRIGHT 2000 CSA
AN 96:48838 LIFESCI

TI Heterologous expression and self-assembly of the **S-layer protein** SbsA of Bacillus stearothermophilus in Escherichia coli
AU Kuen, B.; Sara, M.; **Lubitz, W.**
CS Inst. Mikrobiol. und Genet., Univ. Wien Dr. Bohrg. 9, A-1030 Wien, Austria
SO MOL. MICROBIOL., (1995) vol. 19, no. 3, pp. 495-503.
ISSN: 0950-382X.
DT Journal
FS J
LA English
SL English
AB The cell surface of Bacillus stearothermophilus PV72 is covered by a regular surface layer (S-layer) composed of a single species of protein, SbsA, with a molecular weight of 130 000. Recently, the sequence of the corresponding gene (sbsA) has been determined. The SbsA coding region including the signal sequence was cloned as a polymerase chain reaction (PCR) product into a low-copy-number vector under the transcriptional control of the lambda pL promoter. Expression of sbsA was shown to be thermally inducible from the resulting vector pBK4 in a strain of Escherichia coli expressing the lambda cI857 from the chromosome. As shown by ultrathin sectioning of whole cells and immunogold labelling using SbsA-specific antibodies, expression of sbsA in E. coli led to accumulation of sheet-like self-assembling products of the protein in the cytoplasm. No SbsA protein was detected either in the periplasm or in the supernatant fractions. Long-term expression of sbsA from pBK4, including in the late stationary phase, did not lead to degradation of SbsA.

L6 ANSWER 13 OF 14 EMBASE COPYRIGHT 2000 ELSEVIER SCI. B.V.DUPLICATE 8
AN 94230051 EMBASE
DN 1994230051
TI Sequence analysis of the sbsA gene encoding the 130-kDa surface-layer protein of Bacillus stearothermophilus strain PV72.
AU Kuen B.; Sleytr U.B.; **Lubitz W.**
CS Inst. of Microbiology and Genetics, University of Vienna, Dr. Bohrgasse 9,A-1030 Vienna, Austria
SO Gene, (1994) 145/1 (115-120).
ISSN: 0378-1119 CODEN: GENED6
CY Netherlands
DT Journal; Article
FS 004 Microbiology
029 Clinical Biochemistry
LA English
SL English
AB Bacillus stearothermophilus (Bs) contains a surface-layer (S-layer) protein (SbsA), which forms a hexagonal array on the cell wall. In order to understand the structural/functional relationship of SbsA from Bs PV72, the entire nucleotide (nt) sequence of the sbsA gene was determined from three overlapping fragments. The 3'-end was cloned and expressed in Escherichia coli, whereas the 5'-region was amplified from the genome of Bs PV72 by the polymerase chain reaction using two overlapping fragments. The open reading frame (3684 nt) of sbsA is predicted to encode a protein of 1228 amino acids (aa). The SbsA is synthesized with a leader sequence of 30 aa. The predicted SbsA aa profile was similar to most other sequenced S-layer proteins, containing more acidic than basic aa (pI 5.1) and a very low amount of sulfur-containing aa. Based on aa sequence data, SbsA has weak homology of with the S-layer proteins from B. sphaericus, Rickettsia rickettsii, B. brevis HPD31 and B. brevis 47 (OWP).

L6 ANSWER 14 OF 14 CAPLUS COPYRIGHT 2000 ACS
AN 1995:29574 CAPLUS
DN 122:76093
TI Structural and functional analysis of the S-layer

protein from Bacillus stearothermophilus
AU Kuen, Beatrix; **Lubitz, Werner**; Barton, Geoffrey J.
CS Institute Microbiology and Genetics, University Vienna, Vienna, Austria
SO NATO ASI Ser., Ser. A (1993), 252(ADVANCES IN BACTERIAL PARACRYSTALLINE SURFACE LAYERS), 143-9
CODEN: NALSDJ; ISSN: 0258-1213
DT Journal; General Review
LA English
AB A review and discussion with 16 refs.

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E2	1		SLEYTR UVE B/AU
E3	8	-->	SLEYTR UWE/AU
E4	191		SLEYTR UWE B/AU
E5	15		SLEYTR UWE BERND/AU
E6	1		SLEYTR UWE R/AU
E7	1		SLEYTRE B/AU
E8	1		SLEZ AK J/AU
E9	1		SLEZ AK K/AU
E10	10		SLEZ AK P/AU
E11	1		SLEZ AKOV A E/AU
E12	2		SLEZ L G/AU

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L7 735 "SLEYTR U B"/AU OR "SLEYTR UWE"/AU OR "SLEYTR UWE B"/AU OR "SLEY
TR UWE BERND"/AU

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L8 15 L7 AND (FUSION PROTEIN OR HETEROLOGOUS)

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L9 ANSWER 1 OF 4 EMBASE COPYRIGHT 2000 ELSEVIER SCI. B.V.DUPLICATE 1
AN 2000067008 EMBASE
TI S-layer gene sbsC of Bacillus stearothermaphilus ATCC 12980: Molecular characterization and **heterologous** expression in Escherichia coli.
AU Jarosch M.; Egelseer E.M.; Mattanovich D.; **Sleytr U.B.**; Sara M.
CS M. Sara, Zentrum fur Ultrastrukturforschung, L. Boltzmann Inst. Mol. Nanotechnol., Universitat fur Bodenkultur, 1180 Vienna, Austria. sara@edv1.boku.ac.at
SO Microbiology, (2000) 146/2 (273-281).

Refs: 33
ISSN: 1350-0872 CODEN: MROBEO
CY United Kingdom
DT Journal; Article
FS 004 Microbiology
LA English
SL English
AB The cell surface of Bacillus stearothermophilus ATCC 12980 is completely covered with an oblique S-layer lattice. To investigate sequence identities and a common structure-function relationship in S-layer proteins of different B. stearothermophilus wild-type strains, the nucleotide sequence encoding the S-layer protein SbsC of B. stearothermophilus ATCC 12980 was determined by PCR techniques. The entire sbsC sequence showed an ORF of 3297 bp predicted to encode a protein of 1099 aa with a theoretical molecular mass of 115409 Da and an isoelectric point of 5.73. Primer extension analysis suggested the existence of two promoter regions. Amino acid sequence comparison between SbsC and SbsA, a previously characterized S-layer protein of B. stearothermophilus PV72/p6 which assembles into a hexagonally ordered lattice, revealed an identical secretion signal peptide, 85% identity for the N-terminal regions (aa 31-270) which do not carry any S-layer homologous motifs, but only 21% identity for the rest of the sequences. Affinity studies demonstrated that the N-terminal part of SbsC is necessary for recognition of a secondary cell wall polymer. This was in accordance with results obtained in a previous study for SbsA, thus confirming a common functional principle for the N-terminal parts of both S-layer proteins. The sbsC coding region cloned into the pET3a vector without its own upstream region, the signal sequence and the 3' transcriptional terminator led to stable expression in Escherichia coli.

L9 ANSWER 2 OF 4 EMBASE COPYRIGHT 2000 ELSEVIER SCI. B.V.DUPLICATE 2
AN 97318181 EMBASE
DN 1997318181
TI Factors controlling in vitro recrystallization of the Caulobacter crescentus paracrystalline S-layer.
AU Nomellini J.F.; Kupcu S.; **Sleytr** U.B.; Smit J.
CS J. Smit, Dept. of Microbiology/Immunology, University of British Columbia, 300 - 6174 University Blvd., Vancouver, BC V6T 1Z3, Canada. jsmit@unixg.ubc.ca
SO Journal of Bacteriology, (1997) 179/20 (6349-6354).
Refs: 33
ISSN: 0021-9193 CODEN: JOBAAY
CY United States
DT Journal; Article
FS 004 Microbiology
LA English
SL English
AB The S-layer of Caulobacter is a two-dimensional paracrystalline array on the cell surface composed of a single protein, RsaA. We have established conditions for preparation of stable, soluble protein and then efficient in vitro recrystallization of the purified protein. Efficient recrystallization and long range order could not be obtained with pure protein only, though it was apparent that calcium was required for crystallization. Recrystallization was obtained when lipid vesicles were provided, but only when the vesicles contained the specific species of Caulobacter smooth lipopolysaccharide (SLPS) that previous studies implicated as a requirement for attaching the S- layer to the cell surface. The specific type of phospholipids did not appear critical; phospholipids rather different from those present in Caulobacter membranes

or archaebacterial tetraether lipids worked equally well. The source of LPS was critical; rough and smooth variants of Salmonella typhimurium LPS as well as the rough form of Caulobacter LPS were ineffective. The requirement for calcium ions for recrystallization was further evaluated; strontium ions could substitute for calcium, and to a lesser extent, cobalt, barium, manganese and magnesium ions also stimulated crystallization. On the other hand, nickel and cadmium provided only weak crystallization stimulation, and zinc, copper, iron, aluminum ions, and the monovalent potassium, sodium, and lithium ions were ineffective. The recrystallization could also be reproduced with Langmuir-Blodgett lipid monolayers at an air-water interface. As with the vesicle experiments, this was only successful when SLPS was incorporated into the lipid mix. The best method for RsaA preparation, leading to apparently monomeric protein that was stable for many months, was an extraction with a low pH aqueous solution. We also achieved recrystallization, albeit at lower efficiency, using RsaA protein solubilized by 8 M urea, a method which allows retrieval of protein from inclusions, when expressed as **heterologous** protein in Escherichia coli or when retrieved as shed, precipitated protein from certain mutant caulobacters. In summary, the clarification of recrystallization methods has confirmed the requirement of SLPS as a surface attachment component and suggests that its presence in a membrane-like structure greatly stimulates the extent and quality of S-layer formation. The in vitro approach allowed the demonstration that specific ions are capable of participating in crystallization and now provides an assay for the crystallization potential of modified S-layer proteins, whether they were produced in or can be secreted by caulobacters.

L9 ANSWER 3 OF 4 BIOTECHDS COPYRIGHT 2000 DERWENT INFORMATION LTD
AN 1997-01943 BIOTECHDS
TI Bacterial and archaeal S-layer proteins: structure-function relationships
and their biotechnological applications;
bacterium and archaebacterium crystalline cell surface layer for vaccine, biomimetics, **fusion protein** production, support, biosensor or ultrafiltration membrane, etc.; a review
AU **Sleytr U B**; Sara M
CS Univ.Vienna-Agr.; Ludwig-Boltzmann-Inst.Mol.Nanotechnol.
LO Center for Ultrastructure Research and Ludwig Boltzmann Institute for Molecular Nanotechnology, Universitaet fuer Bodenkultur, Vienna, A-1180 Vienna, Austria.
Email: sleytr@edv.1.boku.ac.at
SO Trends Biotechnol.; (1997) 15, 1, 20-26
CODEN: TRBIDM ISSN: 0167-9430
DT Journal
LA English
AB Crystalline cell surface layers (SLs) composed of planar assemblies of protein or glycoprotein subunits are one of the most commonly observed cell envelope structures of bacteria and archaea. Isolated SL subunits can assemble into monomolecular arrays either in suspension, at liquid-surface interfaces, including lipid films, on liposomes and on solid surfaces. Pores in SLs are of regular size and morphology, and functional groups on the protein lattices are aligned in well-defined positions and orientations. These features of SLs have led to various applications in biotechnology, vaccine development, diagnostics, biomimetics and molecular nanotechnology. Applications of crystalline surface layers include: ultrafiltration membranes; SL microparticles for covalent binding of enzymes for amperometric and optical biosensors; SL supporting structures for Langmuir-Blodgett films and liposomes; SL coated liposomes with immobilized antigens and haptens for vaccination; genetic manipulation of SL proteins for production of self-assembling fusion proteins; matrices for biomineralization; and templates for semiconductor technology. (48 ref)

L9 ANSWER 4 OF 4 EMBASE COPYRIGHT 2000 ELSEVIER SCI. B.V.DUPLICATE 3

AN 76145488 EMBASE
DN 1976145488
TI **Heterologous** reattachment of regular arrays of glycoproteins on bacterial surfaces.
AU **Sleytr U.B.**
CS Strangeways Res. Lab., Cambridge, United Kingdom
SO Nature, (1975) 257/5525 (400-402).
CODEN: NATUAS
DT Journal
FS 029 Clinical Biochemistry
003 Endocrinology
004 Microbiology
LA English

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L1 692 S E1-E3
L2 0 S L1 AND FUSION PROTEIN (5A) BACILLUS
L3 26 S L1 AND (FUSION PROTEIN OR HETEROLOGOUS PROTEIN)
L4 12 DUP REM L3 (14 DUPLICATES REMOVED)
L5 36 S L1 AND S LAYER PROTEIN
L6 14 DUP REM L5 (22 DUPLICATES REMOVED)
E SLEYTR UWE/AU
L7 735 S E1 OR E3 OR E4 OR E5
L8 15 S L7 AND (FUSION PROTEIN OR HETEROLOGOUS)
L9 4 DUP REM L8 (11 DUPLICATES REMOVED)

=> s l7 and s layer protein

L10 213 L7 AND S LAYER PROTEIN

=> s l10 and (fusion protein or heterologous)

L11 6 L10 AND (FUSION PROTEIN OR HETEROLOGOUS)

=> d bib ab 1-6

L11 ANSWER 1 OF 6 EMBASE COPYRIGHT 2000 ELSEVIER SCI. B.V.
AN 2000067008 EMBASE
TI S-layer gene sbsC of Bacillus stearothermaphilus ATCC 12980: Molecular characterization and **heterologous** expression in Escherichia coli.
AU Jarosch M.; Egelseer E.M.; Mattanovich D.; **Sleytr U.B.**; Sara M.
CS M. Sara, Zentrum fur Ultrastrukturforschung, L. Boltzmann Inst. Mol. Nanotechnol., Universitat fur Bodenkultur, 1180 Vienna, Austria. sara@edv1.boku.ac.at
SO Microbiology, (2000) 146/2 (273-281).
Refs: 33
ISSN: 1350-0872 CODEN: MROBEO
CY United Kingdom
DT Journal; Article
FS 004 Microbiology
LA English
SL English
AB The cell surface of Bacillus stearothermophilus ATCC 12980 is completely covered with an oblique S-layer lattice. To investigate sequence identities and a common structure-function relationship in S-layer

proteins of different B. stearothermophilus wild-type strains, the nucleotide sequence encoding the **S-layer protein** SbsC of B. stearothermophilus ATCC 12980 was determined by PCR techniques. The entire sbsC sequence showed an ORF of 3297 bp predicted to encode a protein of 1099 aa with a theoretical molecular mass of 115409 Da and an isoelectric point of 5.73. Primer extension analysis suggested the existence of two promoter regions. Amino acid sequence comparison between SbsC and SbsA, a previously characterized S-**layer protein** of B. stearothermophilus PV72/p6 which assembles into a hexagonally ordered lattice, revealed an identical secretion signal peptide, 85% identity for the N-terminal regions (aa 31-270) which do not carry any S-layer homologous motifs, but only 21% identity for the rest of the sequences. Affinity studies demonstrated that the N-terminal part of SbsC is necessary for recognition of a secondary cell wall polymer. This was in accordance with results obtained in a previous study for SbsA, thus confirming a common functional principle for the N-terminal parts of both S-layer proteins. The sbsC coding region cloned into the pET3a vector without its own upstream region, the signal sequence and the 3' transcriptional terminator led to stable expression in Escherichia coli.

L11 ANSWER 2 OF 6 MEDLINE
AN 2000170659 MEDLINE
DN 20170659
TI S-layer gene sbsC of Bacillus stearothermophilus ATCC 12980: molecular characterization and **heterologous** expression in Escherichia coli.
AU Jarosch M; Egelseer E M; Mattanovich D; **Sleytr U B**; Sara M
CS Zentrum fur Ultrastrukturforschung und Ludwig Boltzmann-Insitut fur Molekulare Nanotechnologie, Universitat fur Bodenkultur, Vienna, Austria.
SO MICROBIOLOGY, (2000 Feb) 146 (Pt 2) 273-81.
Journal code: BXW. ISSN: 1350-0872.
CY ENGLAND: United Kingdom
DT Journal; Article; (JOURNAL ARTICLE)
LA English
FS Priority Journals
OS GENBANK-AF055578
EM 200007
EW 20000701
AB The cell surface of Bacillus stearothermophilus ATCC 12980 is completely covered with an oblique S-layer lattice. To investigate sequence identities and a common structure-function relationship in S-layer proteins of different B. stearothermophilus wild-type strains, the nucleotide sequence encoding the **S-layer protein** SbsC of B. stearothermophilus ATCC 12980 was determined by PCR techniques. The entire sbsC sequence showed an ORF of 3297 bp predicted to encode a protein of 1099 aa with a theoretical molecular mass of 115409 Da and an isoelectric point of 5.73. Primer extension analysis suggested the existence of two promoter regions. Amino acid sequence comparison between SbsC and SbsA, a previously characterized S-**layer protein** of B. stearothermophilus PV72/p6 which assembles into a hexagonally ordered lattice, revealed an identical secretion signal peptide, 85% identity for the N-terminal regions (aa 31-270) which do not carry any S-layer homologous motifs, but only 21% identity for the rest of the sequences. Affinity studies demonstrated that the N-terminal part of SbsC is necessary for recognition of a secondary cell wall polymer. This was in accordance with results obtained in a previous study for SbsA, thus confirming a common functional principle for

the N-terminal parts of both S-layer proteins. The sbsC coding region cloned into the pET3a vector without its own upstream region, the signal sequence and the 3' transcriptional terminator led to stable expression in Escherichia coli.

L11 ANSWER 3 OF 6 BIOSIS COPYRIGHT 2000 BIOSIS
AN 2000:178997 BIOSIS
DN PREV200000178997
TI S-layer gene sbsC of Bacillus stearothermophilus ATCC 12980: Molecular characterization and **heterologous** expression in Escherichia coli.
AU Jarosch, Marina; Egelseer, Eva M.; Mattanovich, Diethard; **Sleytr, Uwe** B.; Sara, Margit (1)
CS (1) Zentrum fur Ultrastrukturforschung und Ludwig Boltzmann-Institut fur Molekulare Nanotechnologie, Universitat fur Bodenkultur, 1180, Vienna Austria
SO Microbiology (Reading), (Feb., 2000) Vol. 146, No. 2, pp. 273-281. ISSN: 1350-0872.
DT Article
LA English
SL English
AB The cell surface of Bacillus stearothermophilus ATCC 12980 is completely covered with an oblique S-layer lattice. To investigate sequence identities and a common structure-function relationship in S-layer proteins of different B. stearothermophilus wild-type strains, the nucleotide sequence encoding the **S-layer protein** SbsC of B. stearothermophilus ATCC 12980 was determined by PCR techniques. The entire sbsC sequence showed an ORF of 3297 bp predicted to encode a protein of 1099 aa with a theoretical molecular mass of 115409 Da and an isoelectric point of 5.73. Primer extension analysis suggested the existence of two promoter regions. Amino acid sequence comparison between SbsC and SbsA, a previously characterized **S-layer protein** of B. stearothermophilus PV72/p6 which assembles into a hexagonally ordered lattice, revealed an identical secretion signal peptide, 85% identity for the N-terminal regions (aa 31-270) which do not carry any S-layer homologous motifs, but only 21% identity for the rest of the sequences. Affinity studies demonstrated that the N-terminal part of SbsC is necessary for recognition of a secondary cell wall polymer. This was in accordance with results obtained in a previous study for SbsA, thus confirming a common functional principle for the N-terminal parts of both S-layer proteins. The sbsC coding region cloned into the pET3a vector without its own upstream region, the signal sequence and the 3' transcriptional terminator led to stable expression in Escherichia coli.

L11 ANSWER 4 OF 6 CAPLUS COPYRIGHT 2000 ACS
AN 2000:148642 CAPLUS
DN 132:330499
TI S-layer gene sbsC of Bacillus stearothermophilus ATCC 12980: molecular characterization and **heterologous** expression in Escherichia coli
AU Jarosch, Marina; Egelseer, Eva M.; Mattanovich, Diethard; **Sleytr, Uwe B.**; Sara, Margit
CS Zentrum fur Ultrastrukturforschung und Ludwig Boltzmann-Institut fur Molekulare Nanotechnologie, Zentrum fur Ultrastrukturforschung und Ludwig Boltzmann-Institut fur Molekulare Nanotechnologie, Universitat fur Bodenkultur, Vienna, 1180, Austria
SO Microbiology (Reading, U. K.) (2000), 146(2), 273-281
CODEN: MROBEO; ISSN: 1350-0872
PB Society for General Microbiology
DT Journal

LA English
AB The cell surface of Bacillus stearothermophilus ATCC 12980 is completely covered with an oblique S-layer lattice. To investigate sequence identities and a common structure-function relationship in S-layer proteins of different B. stearothermophilus wild-type strains, the nucleotide sequence encoding the **S-layer protein** SbsC of B. stearothermophilus ATCC 12980 was detd. by PCR techniques. The entire sbsC sequence showed an ORF of 3297 bp predicted to encode a protein of 1099 aa with a theor. mol. mass of 115409 Da and an isoelec. point of 5.73. Primer extension anal. suggested the existence of two promoter regions. Amino acid sequence comparison between SbsC and SbsA, a previously characterized **S-layer protein** of B. stearothermophilus PV72/p6 which assembles into a hexagonally ordered lattice, revealed an identical secretion signal peptide, 85% identity for the N-terminal regions (aa 31-270) which do not carry any S-layer homologous motifs, but only 21% identity for the rest of the sequences. Affinity studies demonstrated that the N-terminal part of SbsC is necessary for recognition of a secondary cell wall polymer. This was in accordance with results obtained in a previous study for SbsA, thus confirming a common functional principle for the N-terminal parts of both S-layer proteins. The sbsC coding region cloned into the pET3a vector without its own upstream region, the signal sequence and the 3' transcriptional terminator led to stable expression in Escherichia coli.
RE.CNT 35
RE
(1) Adachi, T; J Bacteriol 1989, V171, P1010 CAPLUS
(2) Altschul, S; Nucleic Acids Res 1997, V25, P3389 CAPLUS
(3) Boot, H; J Bacteriol 1996, V178, P5388 CAPLUS
(4) Brechtel, E; J Bacteriol 1999, V181, P5017 CAPLUS
(5) Chauvaux, S; J Bacteriol 1999, V181, P2455 CAPLUS
ALL CITATIONS AVAILABLE IN THE RE FORMAT

L11 ANSWER 5 OF 6 CAPLUS COPYRIGHT 2000 ACS
AN 1997:677556 CAPLUS
DN 127:356710
TI Factors controlling in vitro recrystallization of the Caulobacter crescentus paracrystalline S-layer
AU Nomellini, John F.; Kupcu, Seta; **Sleytr, Uwe B.**; Smit, John
CS Department of Microbiology and Immunology, University of British Columbia, Vancouver, BC, V6T 1Z3, Can.
SO J. Bacteriol. (1997), 179(20), 6349-6354
CODEN: JOBAAY; ISSN: 0021-9193
PB American Society for Microbiology
DT Journal
LA English
AB The S-layer of Caulobacter is a two-dimensional paracryst. array on the cell surface composed of a single protein, RsaA. We established conditions for prepn. of stable, sol. protein and then efficient in vitro recrystn. of the purified protein. Efficient recrystn. and long-range order could not be obtained with pure protein only, though it was apparent that calcium was required for crystn. Recrystn. was obtained when lipid vesicles were provided, but only when the vesicles contained the specific species of Caulobacter smooth lipopolysaccharide (SLPS) that previous studies implicated as a requirement for attaching the S-layer to the cell surface. The specific type of phospholipids did not appear crit.; phospholipids rather different from those present in Caulobacter membranes or archaebacterial tetraether lipids worked equally well. The source of LPS was crit.; rough and smooth variants of Salmonella typhimurium LPS as

well as the rough form of Caulobacter LPS were ineffective. The requirement for calcium ions for recrystn. was further evaluated: strontium ions could substitute for calcium, and to a lesser extent, cobalt, barium, manganese, and magnesium ions also stimulated crystn. On the other hand, nickel and cadmium provided only weak crystn. stimulation, and zinc, copper, iron, aluminum ions, and monovalent potassium, sodium, and lithium ions were ineffective. Recrystn. could also be reproduced with Langmuir-Blodgett lipid monolayers at an air-water interface. As with the vesicle expts., this was only successful when SLPS was incorporated into the lipid mix. The best method for RsaA prepn., leading to apparently monomeric protein that was stable for many months, was extn. with a low-pH aq. soln. We also achieved recrystn., albeit at lower efficiency, by using RsaA protein solubilized by 8M urea, a method which allows retrieval of protein from inclusions, when expressed as **heterologous** protein in Escherichia coli or when retrieved as shed, pptd. protein from certain mutant caulobacters. Thus, clarification of recrystn. methods confirmed the requirement of SLPS as a surface attachment component and suggests that its presence in a membrane-like structure greatly stimulates the extent and quality of S-layer formation. The in vitro approach allowed the demonstration that specific ions are capable of participating in crystn. and now provides an assay for the crystn. potential of modified S-layer proteins, whether they were produced in or can be secreted by caulobacters.

L11 ANSWER 6 OF 6 LIFESCI COPYRIGHT 2000 CSA
AN 2000:82238 LIFESCI
TI S-layer gene sbsC of Bacillus stearothermophilus ATCC 12980: molecular characterization and **heterologous** expression in Escherichia coli
AU Jarosch, M.; Egelseer, E.M.; Mattanovich, D.; **Sleytr, U.B.**; Sara, M.
CS Zentrum fur Ultrastrukturforschung und Ludwig Boltzmann-Institut fur Molekulare Nanotechnologie, Universitat fur Bodenkultur, 1180 Vienna, Austria
SO Microbiology, (20000200) vol. 146, no. 2, pp. 273-281.
ISSN: 1350-0872.
DT Journal
FS J
LA English
SL English
AB The cell surface of Bacillus stearothermophilus ATCC 12980 is completely covered with an oblique S-layer lattice. To investigate sequence identities and a common structure--function relationship in S- layer proteins of different B. stearothermophilus wild-type strains, the nucleotide sequence encoding the **S-layer protein** SbsC of B. stearothermophilus ATCC 12980 was determined by PCR techniques. The entire sbsC sequence showed an ORF of 3297 bp predicted to encode a protein of 1099 aa with a theoretical molecular mass of 115409 Da and an isoelectric point of 5.73. Primer extension analysis suggested the existence of two promoter regions. Amino acid sequence comparison between SbsC and SbsA, a previously characterized S-**layer protein** of B. stearothermophilus PV72/p6 which assembles into a hexagonally ordered lattice, revealed an identical secretion signal peptide, 85% identity for the N-terminal regions (aa 31--270) which do not carry any S-layer homologous motifs, but only 21% identity for the rest of the sequences. Affinity studies demonstrated that the N-terminal part of SbsC is necessary for recognition of a secondary cell wall polymer. This was in accordance with results obtained in a previous study for SbsA, thus confirming a common functional principle for

the N- terminal parts of both S-layer proteins. The sbsC coding region cloned into the pET3a vector without its own upstream region, the signal sequence and the 3' transcriptional terminator led to stable expression in
Escherichia coli.

=> e kuen beatrix/au

```
E1          38     KUEN B/AU
E2           1     KUEN BEA/AU
E3          21 --> KUEN BEATRIX/AU
E4           2     KUEN C L/AU
E5           1     KUEN C Y/AU
E6           1     KUEN CHAN LAU/AU
E7           2     KUEN CHI FOUN/AU
E8           1     KUEN CHOY K/AU
E9           2     KUEN D/AU
E10          7     KUEN DAVID A/AU
E11          4     KUEN DAVID ARTHUR/AU
E12          1     KUEN DIETRICH/AU
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=> s e1-e3

L12 60 ("KUEN B"/AU OR "KUEN BEA"/AU OR "KUEN BEATRIX"/AU)

=> s l12 and (fusion protein or heterologous)

L13 5 L12 AND (FUSION PROTEIN OR HETEROLOGOUS)

=> d bib ab 1-5

L13 ANSWER 1 OF 5 EMBASE COPYRIGHT 2000 ELSEVIER SCI. B.V.
AN 96051831 EMBASE
DN 1996051831
TI **Heterologous** expression and self-assembly of the S-layer protein SbsA of Bacillus stearothermophilus in Escherichia coli.
AU **Kuen** B.; Sara M.; Lubitz W.
CS Institut fur Mikrobiologie/Genetik, Universitat Wien, Dr. Bohrg 9,A-1030 Wien, Austria
SO Molecular Microbiology, (1996) 19/3 (495-503).
ISSN: 0950-382X CODEN: MOMIEE
CY United Kingdom
DT Journal; Article
FS 004 Microbiology
LA English
SL English
AB The cell surface of Bacillus stearothermophilus PV72 is covered by a regular surface layer (S-layer) composed of a single species of protein, SbsA, with a molecular weight of 130000. Recently, the sequence of the corresponding gene (sbsA) has been determined. The SbsA coding region including the signal sequence was cloned as a polymerase chain reaction (PCR) product into a low-copy-number vector under the transcriptional control of the .lambda.pL promoter. Expression of sbsA was shown to be thermally inducible from the resulting vector pBK4 in a strain of Escherichia coli expressing the .lambda.cI857 from the chromosome. As shown by ultrathin sectioning of whole cells and immunogold labelling using SbsA-specific antibodies, expression of sbsA in E. coli led to accumulation of sheet-like self-assembling products of the protein in the cytoplasm. No SbsA protein was detected either in the periplasm or in the supernatant fractions. Long-term expression of sbsA from pBK4, including in the late stationary phase, did not lead to degradation of SbsA.

L13 ANSWER 2 OF 5 MEDLINE

AN 96228698 MEDLINE
DN 96228698
TI **Heterologous** expression and self-assembly of the S-layer protein SbsA of Bacillus stearothermophilus in Escherichia coli.
AU **Kuen B**; Sara M; Lubitz W
CS Institut fur Mikrobiologie und Genetik, Universitat, Austria.. oetzi@gem.univie.ac.at
SO MOLECULAR MICROBIOLOGY, (1996 Feb) 19 (3) 495-503. Journal code: MOM. ISSN: 0950-382X.
CY ENGLAND: United Kingdom
DT Journal; Article; (JOURNAL ARTICLE)
LA English
FS Priority Journals
EM 199612
AB The cell surface of Bacillus stearothermophilus PV72 is covered by a regular surface layer (S-layer) composed of single species of protein, SbsA, with a molecular weight of 130,000. Recently, the sequence of the corresponding gene (sbsA) has been determined. The SbsA coding region including the signal sequence was cloned as a polymerase chain reaction (PCR) product into a low-copy-number vector under the transcriptional control of the lambda pL promoter. Expression of sbsA was shown to be thermally inducible from the resulting vector pBK4 in a strain of Escherichia coli expressing the lambda c1857 from the chromosome. As shown by ultrathin sectioning of whole cells and immunogold labelling using SbsA- specific antibodies, expression of sbsA in E. coli led to accumulation of sheet-like self-assembling products of the protein in the cytoplasm. No SbsA protein was detected either in the periplasm or in the supernatant fractions. Long-term expression of sbsA from pBK4, including in the late stationary phase, did not lead to degradation of SbsA.

L13 ANSWER 3 OF 5 BIOSIS COPYRIGHT 2000 BIOSIS
AN 1996:154851 BIOSIS
DN PREV199698726986
TI **Heterologous** expression and self-assembly of the S-layer protein SbsA of Bacillus stearothermophilus in Escherichia coli.
AU **Kuen, Beatrix (1)**; Sara, Margit; Lubitz, Werner
CS (1) Inst. Mikrobiologie und Genetik, Univ. Wein Dr. Bohrg. 9, A-1030 Wien Austria
SO Molecular Microbiology, (1996) Vol. 19, No. 3, pp. 495-503. ISSN: 0950-382X.
DT Article
LA English
AB The cell surface of Bacillus stearothermophilus PV72 is covered by a regular surface layer (S-layer) composed of a single species of protein, SbSA, with a molecular weight of 130000. Recently, the sequence of the corresponding gene (sbsA) has been determined. The SbsA coding region including the signal sequence was cloned as a polymerase chain reaction (PCR) product into a low-copy-number vector under the transcriptional control of the lambda-pL promoter. Expression of sbsA was shown to be thermally inducible from the resulting vector pBK4 in a strain of Escherichia coli expressing the lambda-cI857 from the chromosome. As shown by ultrathin sectioning of whole cells and immunogold labelling using SbsA-specific antibodies, expression of sbsA in E. coli led to accumulation of sheet-like self-assembling products of the protein in the cytoplasm. No SbsA protein was detected either in the periplasm or in the supernatant fractions. Long-term expression of sbsA from pBK4, including in the late stationary phase, did not lead to degradation of SbsA.

L13 ANSWER 4 OF 5 CAPLUS COPYRIGHT 2000 ACS
AN 1996:120270 CAPLUS
DN 124:195218
TI **Heterologous** expression and self-assembly of the S-layer protein SbsA of Bacillus stearothermophilus in Escherichia coli

AU **Kuen, Beatrix**; Sara, Margit; Lubitz, Werner
CS Inst. Mikrobiologie Genetik, Universitaet Vienna, Vienna, A-1030, Austria
SO Mol. Microbiol. (1996), 19(3), 495-503
CODEN: MOMIEE; ISSN: 0950-382X
DT Journal
LA English
AB The cell surface of Bacillus stearothermophilus PV72 is covered by a regular surface layer (S-layer) composed of single species of protein, SbsA, with a mol. wt. of 130 000. Recently, the sequence of the corresponding gene (sbsA) has been detd. The SbsA coding region including the signal sequence was cloned as a polymerase chain reaction (PCR) product into a low-copy-no. vector under the transcriptional control of the .lambda.pL promoter. Expression of sbsA was shown to be thermally inducible from the resulting vector pBK4 in a strain of Escherichia coli expressing the .lambda.c1857 from the chromosome. As shown by ultrathin sectioning of whole cells and immunogold labeling using SbsA-specific antibodies expression of sbsA in E. coli led to accumulation of sheet-like self-assembling products of the protein in the cytoplasm. No SbsA protein was detected either in the periplasm or in the supernatant fractions. Long-term expression of sbsA from pBK4, including in the late stationary phase, did not lead to degrdn. of SbsA.

L13 ANSWER 5 OF 5 LIFESCI COPYRIGHT 2000 CSA
AN 96:48838 LIFESCI
TI **Heterologous** expression and self-assembly of the S-layer protein SbsA of Bacillus stearothermophilus in Escherichia coli
AU **Kuen**, B.; Sara, M.; Lubitz, W.
CS Inst. Mikrobiol. und Genet., Univ. Wien Dr. Bohrg. 9, A-1030 Wien, Austria
SO MOL. MICROBIOL., (1995) vol. 19, no. 3, pp. 495-503.
ISSN: 0950-382X.
DT Journal
FS J
LA English
SL English
AB The cell surface of Bacillus stearothermophilus PV72 is covered by a regular surface layer (S-layer) composed of a single species of protein, SbsA, with a molecular weight of 130 000. Recently, the sequence of the corresponding gene (sbsA) has been determined. The SbsA coding region including the signal sequence was cloned as a polymerase chain reaction (PCR) product into a low-copy-number vector under the transcriptional control of the lambda pL promoter. Expression of sbsA was shown to be thermally inducible from the resulting vector pBK4 in a strain of Escherichia coli expressing the lambda c1857 from the chromosome. As shown by ultrathin sectioning of whole cells and immunogold labelling using SbsA-specific antibodies, expression of sbsA in E. coli led to accumulation of sheet-like self-assembling products of the protein in the cytoplasm. No SbsA protein was detected either in the periplasm or in the supernatant fractions. Long-term expression of sbsA from pBK4, including in the late stationary phase, did not lead to degradation of SbsA.

=> s l12 and s layer protein

L14 41 L12 AND S LAYER PROTEIN

=> dup rem l14

PROCESSING COMPLETED FOR L14
L15 14 DUP REM L14 (27 DUPLICATES REMOVED)

=> d bib ab 1-14

L15 ANSWER 1 OF 14 EMBASE COPYRIGHT 2000 ELSEVIER SCI. B.V.DUPLICATE 1
AN 2000287774 EMBASE
TI The transposable element IS4712 prevents S-layer gene (sbsA) expression in
Bacillus stearothermophilus and also affects the synthesis of altered surface layer proteins.
AU Scholz H.; Hummel S.; Witte A.; Lubitz W.; **Kuen B.**
CS H. Scholz, Institute of Animal Hygiene, Public Veterinary Health, An den Tierkliniken 43, 04103 Leipzig, Germany. scholz@vetmed.uni-leipzig.de
SO Archives of Microbiology, (2000) 174/1-2 (97-103).
Refs: 19
ISSN: 0302-8933 CODEN: AMICCW
PUI S002030000181
CY Germany
DT Journal; Article
FS 004 Microbiology
029 Clinical Biochemistry
LA English
SL English
AB Cell surface (**S**)-**layer protein** synthesis in Bacillus stearothermophilus PV72/p6 is blocked when cells are grown at elevated temperature. From a culture exhibiting the S-layer-negative phenotype, the S-layer deficient mutant T5 (SbsA-) was isolated. Genetic analysis of the S-layer-encoding gene (sbsA) of mutant T5 revealed an insertion element (IS4712) integrated into the upstream regulatory region of the S-layer gene, thereby blocking sbsA transcription. The insertion element consists of 1371 base pairs which are flanked by two perfect inverted terminal repeats. Sequence similarity to other transposases of the IS4 family was detected. DNA-DNA hybridizations demonstrated that multiple homologues of IS4712 were also present within the genomes of several other thermophilic bacillus isolates. Attempts to isolate SbsA+ revertants failed. Instead, cells with altered surface proteins were detected. The synthesis of the altered S-layer proteins was correlated with the presence of IS4712 along with the occurrence of deletions in the sbsA coding region. Furthermore imprecise excision of IS4712 was detected.
This work demonstrated that B. stearothermophilus is able to express at least four different S-layer proteins and that blocking of sbsA transcription by the insertion element IS4712 is associated with the expression of altered surface proteins.

L15 ANSWER 2 OF 14 BIOTECHDS COPYRIGHT 2000 DERWENT INFORMATION LTD
AN 1999-11466 BIOTECHDS
TI Extended recombinant bacterial ghost system;
ghost cell production and foreign gene and antigen expression for use as a recombinant combination vaccine (conference paper)
AU Lubitz W; Witte A; Eko F O; Kamal M; Jechlinger W; Brand E; Marchart J; Haidinger W; Huter V; Felnerova D; Stralis-Alves N; Lechleitner S; Melzer H; Szostak M P; Resch S; Nader H; **Kuen B**; Mayr B; Mayrhofer P; Geretschlager R; Haslberger A; Hensel A
CS Univ.Vienna-Inst.Microbiol.Genet.; EVAX-Technol.; Univ.Leipzig-Inst.Anim.Hyg.Vet.Public-Health
LO Institute of Microbiology and Genetics, University of Vienna, Dr. Bohrgasse 9, A-1030 Vienna, Austria.
Email: oldfox@gem.univie.ac.at
SO J.Biotechnol.; (1999) 73, 2-3, 261-73
CODEN: JBITD4 ISSN: 0168-1656
New Approaches in Vaccine Development 1997, Australian Society of Biotechnology, Vienna, Austria, 1997.
DT Journal
LA English
AB Controlled expression of cloned PhiX174 gene E in Gram-negative bacteria

results in lysis of the bacteria by formation of an E-specific transmembrane tunnel structure built through the cell envelope complex. These bacterial ghosts from a variety of bacteria were used as non-living candidate vaccines. In a recombinant ghost system, the desired foreign proteins are attached to the inside of the inner membrane as fusions with specific anchor sequences. Because the ghosts have a sealed periplasmic and the proteins can be exported into this space the capacity of the ghost or recombinant ghost systems can be vastly extended, therefore making them capable carriers of foreign antigens. The recombinant ghosts can also express **S-layer protein** (shell-like structure), which can carry foreign gene epitopes, which further extends the possibilities of ghost carriers. The ghost also have inherent adjuvant properties, so they can be used as adjuvants in combination with subunit vaccines. There is no limitations on the size of foreign antigens which can be inserted into the ghosts and so they may be used as adjuvant free combination vaccines. (32 ref)

L15 ANSWER 3 OF 14 EMBASE COPYRIGHT 2000 ELSEVIER SCI. B.V.DUPLICATE 2
AN 1999088729 EMBASE
TI Self-assembly product formation of the Bacillus stearothermophilus PV72/p6 **S-layer protein** SbsA in the course of autolysis of Bacillus subtilis.
AU Howorka S.; Sara M.; Lubitz W.; **Kuen B.**
CS B. Kuen, Institut Mikrobiologie and Genetik, Universitat Wien, Dr. Bohrgasse 9, A-1030 Vienna, Austria. oetzi@gem.univie.ac.at
SO FEMS Microbiology Letters, (1999) 172/2 (187-196).
Refs: 20
ISSN: 0378-1097 CODEN: FMLED7
PUI S 0378-1097(99)00040-3
CY Netherlands
DT Journal; Article
FS 004 Microbiology
LA English
SL English
AB In order to achieve high level expression and to study the release of a protein capable of self-assembly, the gene encoding the crystalline cell surface (**S-layer**) **protein** SbsA of Bacillus stearothermophilus PV72/p6, including its signal sequence, was cloned and expressed in Bacillus subtilis. To obtain high level expression, a tightly regulated, xylose-inducible, stably replicating multicopy-plasmid vector was constructed. After induction of expression, the **S-layer protein** made up about 15% of the total cellular protein content, which was comparable to the SbsA content of B. stearothermophilus PV72/p6 cells. During all growth stages, SbsA was poorly secreted to the ambient cellular environment by B. subtilis. Extraction of whole cells with guanidine hydrochloride showed that in late stationary growth phase cells 65% of the synthesised SbsA was retained in the peptidoglycan-containing layer, indicating that the rigid cell wall layer was a barrier for efficient SbsA secretion. Electron microscopic investigation revealed that SbsA release from the peptidoglycan-containing layer started in the late stationary growth phase at distinct sites at the cell surface leading to the formation of extracellular self-assembly products which did not adhere to the cell wall surface. In addition, intracellular sheet-like SbsA self-assembly products which followed the curvature of the cell became visible in partly lysed cells. Intracellularly formed self-assembly products remained intact even after

complete lysis of the rigid cell envelope layer. Copyright (C) 1999 Federation of European Microbiological Societies.

L15 ANSWER 4 OF 14 BIOTECHDS COPYRIGHT 2000 DERWENT INFORMATION LTD
AN 1997-11103 BIOTECHDS
TI Preparation of S-layer proteins by expressing sbs-A gene in Gram-negative bacterium;
for use as e.g. vaccine or adjuvant
AU Lubitz W; Sleytr U; **Kuen B**
PA Lubitz W; Sleytr U
LO Vienna, Austria.
PI DE 19603649 7 Aug 1997
AI DE 1996-1003649 1 Feb 1996
PRAI DE 1996-1003649 1 Feb 1996
DT Patent
LA German
OS WPI: 1997-394558 [37]
AB A new method for the preparation of **S-layer protein** (I) involves transforming a Gram-negative prokaryote, preferably Escherichia coli, with a nucleic acid encoding (I) contained on a vector, and culturing the transformed cells. The nucleic acid may contain one or more inserts, preferably encoding Cys residues, regions with many charged amino acids or Tyr, DNA-or metal-binding epitopes, immune, allergenic or antigenic epitopes, streptavidin, enzymes or cytokine- or antibody-binding proteins. (I) is useful as a recombinant vaccine or adjuvant, especially when combined with a bacterial ghost that may contain additional epitopes in its membrane. Other uses, depending on the inserted protein, include (a) universal adjuvant for biotinylated reactants for immunological or hybridization assays, (b) induction of immune responses, (c) reagent for removing cytokine or toxin from serum, (d) molecular spinning nozzle and (e) molecular laser. When expressed in Gram-negative cells, (I) is produced in the form of monomolecular layers rather than as inclusion bodies as in Gram-positive bacteria. (31pp)

L15 ANSWER 5 OF 14 CAPLUS COPYRIGHT 2000 ACS
AN 1997:536912 CAPLUS
DN 127:201021
TI Expression of S-layer proteins in Gram-negative bacteria and recombinant chimeric S-layer proteins for use as vaccines
IN Lubitz, Werner; Sleytr, Uwe; **Kuen, Beatrix**; Truppe, Michaela; Howorka, Stefan; Resch, Stepanka; Schroll, Gerhard; Sara, Margit
PA Lubitz, Werner, Austria; Sleytr, Uwe; Kuen, Beatrix; Truppe, Michaela; Howorka, Stefan; Resch, Stepanka; Schroll, Gerhard; Sara, Margit
SO PCT Int. Appl., 65 pp.
CODEN: PIXXD2
DT Patent
LA German
FAN.CNT 1

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
PI WO 9728263	A1	19970807	WO 1997-EP432	19970131
W: AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GE, HU, IL, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, TJ, TM, TR, TT, UA, UG, US, UZ, VN, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM				
RW: KE, LS, MW, SD, SZ, UG, AT, BE, CH, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, BF, BJ, CF, CG, CI, CM, GA, GN, ML, MR, NE, SN, TD, TG				
DE 19603649	A1	19970807	DE 1996-19603649	19960201
CA 2245584	AA	19970807	CA 1997-2245584	19970131
AU 9717203	A1	19970822	AU 1997-17203	19970131

AU 713999 B2 19991216
EP 882129 A1 19981209 EP 1997-904360 19970131
R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, SE, MC, PT, IE, FI
CN 1213402 A 19990407 CN 1997-192940 19970131
JP 2000503850 T2 20000404 JP 1997-527307 19970131
PRAI DE 1996-19603649 19960201
WO 1997-EP432 19970131
AB The invention concerns processess for the recombinant prepn. of S-layer proteins in Gram-neg. host cells. In addn., the nucleotide sequence of a new S-layer gene, the sbsB gene of Bacillus stearothermophilus, and a process for prepn. of modified S-layer proteins is disclosed.
Recombinant
Escherichia coli expressing the sbsA gene of B. stearothermophilus and chimeric sbsA genes encoding SbsA into which various peptides, proteins and enzymes have been inserted were prepd. and cultured to produce the proteins.

L15 ANSWER 6 OF 14 EMBASE COPYRIGHT 2000 ELSEVIER SCI. B.V.DUPLICATE 3
AN 97071257 EMBASE
DN 1997071257
TI Molecular characterization of the Bacillus stearothermophilus PV72 S-layer gene sbsB induced by oxidative stress.
AU **Kuen B.**; Koch A.; Asenbauer E.; Sara M.; Lubitz W.
CS B. Kuen, Inst. of Microbiology and Genetics, Biocenter Vienna, Dr. Bohrg. 9, 1030 Vienna, Austria. oetzi@gem.univie.ac.at
SO Journal of Bacteriology, (1997) 179/5 (1664-1670).
Refs: 42
ISSN: 0021-9193 CODEN: JOBAAY
CY United States
DT Journal; Article
FS 004 Microbiology
LA English
SL English
AB **S-layer protein** variation from a hexagonally ordered (SbsA; 130 kDa) to a obliquely ordered (SbsB; 98 kDa) protein in Bacillus stearothermophilus PV72 is mediated by an increased oxygen supply. To elucidate the molecular basis of **S-layer protein** variation in B. stearothermophilus PV72, the sbsB gene, ceding for the 98-kDa protein, was cloned by means of inverse PCR technology and sequenced. The sbsB coding region cloned in pUC18 was expressed in Escherichia coli, without its own regulatory upstream sequences but with its putative transcriptional terminator. The reading frame of sbsB (2,760 nucleotides) is predicted to encode a protein of 920 amino acids, including the signal sequence. Amino acid sequence comparison
of SbsA and SbsB did not reveal any significant homology. The expression of sbsB in E. coli resulted in an accumulation of SbsB self-assembly products in the cytoplasm.

L15 ANSWER 7 OF 14 EMBASE COPYRIGHT 2000 ELSEVIER SCI. B.V.DUPLICATE 4
AN 97267948 EMBASE
DN 1997267948
TI IV. Molecular biology of S-layers.
AU Bahl H.; Scholz H.; Bayan N.; Chami M.; Leblon G.; Gulik-Krzywicki T.; Shechter E.; Fouet A.; Mesnage S.; Tosi-Couture E.; Gounon P.; Mock M.; De
Macario E.C.; Macario A.J.L.; Fernandez-Herrero L.A.; Olabarria G.; Berenguer J.; Blaser M.J.; **Kuen B.**; Lubitz W.; Sara M.; Pouwels P.H.; Kolen C.P.A.M.; Boot H.J.; Palva A.; Truppe M.; Howorka S.; Schroll G.; Lechleitner S.; Resch S.
CS Dr. N. Bayan, Laboratoire des Biomembranes, URA 1116 CNRS, Universite de Paris-Sud, F-91405 Orsay, France
SO FEMS Microbiology Reviews, (1997) 20/1-2 (47-98).
Refs: 197

ISSN: 0168-6445 CODEN: FMREE4
PUI S 0168-6445(97)00050-8
CY Netherlands
DT Journal; General Review
FS 004 Microbiology
LA English
SL English
AB In this chapter we report on the molecular biology of crystalline surface layers of different bacterial groups. The limited information indicates that there are many variations on a common theme. Sequence variety, antigenic diversity, gene expression, rearrangements, influence of environmental factors and applied aspects are addressed. There is considerable variety in the S-layer composition, which was elucidated by sequence analysis of the corresponding genes. In Corynebacterium glutamicum one major cell wall protein is responsible for the formation of a highly ordered, hexagonal array. In contrast, two abundant surface proteins form the S-layer of Bacillus anthracis. Each protein possesses three S-layer homology motifs and one protein could be a virulence factor.
The antigenic diversity and ABC transporters are important features, which have been studied in methanogenic archaea. The expression of the S-layer components is controlled by three genes in the case of Thermus thermophilus. One has repressor activity on the S-layer gene promoter, the second codes for the **S-layer protein**. The rearrangement by reciprocal recombination was investigated in Campylobacter fetus. 7-8 S-layer proteins with a high degree of homology at the 5' and 3' ends were found. Environmental changes influence the surface properties of Bacillus stearothermophilus. Depending on oxygen supply, this species produces different S-layer proteins. Finaly, the molecular bases for some applications are discussed. Recombinant S-layer fusion proteins have been designed for biotechnology.

L15 ANSWER 8 OF 14 EMBASE COPYRIGHT 2000 ELSEVIER SCI. B.V.DUPLICATE 5
AN 96106121 EMBASE
DN 1996106121
TI Dynamics in oxygen-induced changes in **S-layer protein** synthesis from Bacillus stearothermophilus PV72 and the S-layer-deficient variant T5 in continuous culture and studies of the cell wall composition.
AU Sara M.; **Kuen B.**; Mayer H.F.; Mandl F.; Schuster K.C.; Sleytr U.B.
CS Zentrum fur Ultrastrukturforschung, Universitat fur Bodenkultur, Gregor-Mendelstr. 33,1180 Vienna, Austria
SO Journal of Bacteriology, (1996) 178/7 (2108-2117).
ISSN: 0021-9193 CODEN: JOBAAY
CY United States
DT Journal; Article
FS 004 Microbiology
LA English
SL English
AB Stable synthesis of the hexagonally ordered (p6) **S-layer protein** from the wild-type strain of Bacillus stearothermophilus PV72 could be achieved in continuous culture on complex medium only under oxygen-limited conditions when glucose was used as the sole carbon source.
Depending on the adaptation of the wild-type strain to low oxygen supply, the dynamics in oxygen-induced changes in **S-layer protein** synthesis was different when the rate of aeration was increased to a level that allowed dissimilation of amino acids. If oxygen supply was increased at the beginning of continuous culture, synthesis of the p6 **S-layer protein** from the wild-type

strain (encoded by the sbsA gene) was immediately stopped and replaced by that of a new type of **S-layer protein** (encoded by the sbsB gene) which assembled into an oblique (p2) lattice. In cells adapted to prolonged low oxygen supply, first, low-level p2 **S-layer protein** synthesis and second, synchronous synthesis of comparable amounts of both types of S-layer proteins could be induced by stepwise increasing the rate of aeration. The time course of changes in **S-layer protein** synthesis was followed up by immunogold labelling of whole cells. Synthesis of the p2 **S- layer protein** could also be induced in the p6-deficient variant T5. Hybridization data obtained by applying the radiolabelled N-terminal and C- terminal sbsA fragments and the N-terminal sbsB fragment to the genomic DNA of all the three organisms indicated that changes in **S-layer protein** synthesis were accompanied by chromosomal rearrangement. Chemical analysis of peptidoglycan-containing sacculi and extraction and recrystallization experiments revealed that at least for the wild-type strain, a cell wall polymer consisting of N-acetylglucosamine and glucose is responsible for binding of the p6 **S-layer protein** to the rigid cell wall layer.

L15 ANSWER 9 OF 14 EMBASE COPYRIGHT 2000 ELSEVIER SCI. B.V.DUPLICATE 6
AN 96114000 EMBASE
DN 1996114000
TI Description of Bacillus thermoaerophilus sp. nov., to include sugar beet isolates and Bacillus brevis ATCC 12990.
AU Meier-Stauffer K.; Busse H.-J.; Rainey F.A.; Burghardt J.; Scheberl A.; Hollaus F.; **Kuen B.**; Makristathis A.; Sleytr U.B.; Messner P.
CS Zentrum fur Ultrastrukturforschung, Universitat fur Bodenkultur, Gregor-Mendel-Str. 33,A-1180 Vienna, Austria
SO International Journal of Systematic Bacteriology, (1996) 46/2 (532-541). ISSN: 0020-7713 CODEN: IJSBA8
CY United States
DT Journal; Article
FS 004 Microbiology
LA English
SL English
AB Isolates of thermophilic bacteria obtained from an Austrian beet sugar factory were screened by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) and freeze-fracture electron microscopy for the presence of glycosylated crystalline cell surface layers (S-layers). On the basis of similarities in the protein band patterns on SDS-PAGE gels and the lattice geometry of the S-layers as revealed by electron micrographs, the 31 isolates which we studied were clustered into five groups (groups I to V) and several strains which exhibited no common characteristics (group 0). We found that the organisms belonging to groups I to III had glycosylated S-layer proteins, but the highest carbohydrate contents were observed in group III organisms. Partial sequencing of the 16S ribosomal DNAs of selected representative strains of each group revealed that the group I, II, IV, and V isolates and the few group 0 strains were different from the group III strains. The results of DNA-DNA hybridization experiments, SDS-PAGE, and an analysis of polar lipids demonstrated that group Ill isolates LA19-91, L420- 91(T) (T = type strain), and L438-91 belong to the same species. We chose the group III organism Bacillus sp. strain L420-9(T) for further analysis because of the high carbohydrate content of its **S-layer protein**. The taxonomic position of this isolate was determined by using a polyphasic approach. Phenotypic, chemotaxonomic, and genomic analyses revealed that strains L420- 91(T), L419-91, and L438-91 represent a new Bacillus species. We observed high levels of similarity between

these strains and Bacillus brevis ATCC 12990, which also had a glycosylated **S-layer protein**. Our results show that strains L420-91(T), L419-91, and L438-91 and B. brevis ATCC 12990 belong to the same species and that this species is a new Bacillus species, which we name Bacillus thermoaerophilus. The type strain of this species is strain L420-91 (= DSM 10154).

L15 ANSWER 10 OF 14 EMBASE COPYRIGHT 2000 ELSEVIER SCI. B.V.DUPLICATE 7
AN 96051831 EMBASE
DN 1996051831
TI Heterologous expression and self-assembly of the **S-layer protein** SbsA of Bacillus stearothermophilus in Escherichia coli.
AU **Kuen B.**; Sara M.; Lubitz W.
CS Institut fur Mikrobiologie/Genetik, Universitat Wien, Dr. Bohrg 9,A-1030 Wien, Austria
SO Molecular Microbiology, (1996) 19/3 (495-503).
ISSN: 0950-382X CODEN: MOMIEE
CY United Kingdom
DT Journal; Article
FS 004 Microbiology
LA English
SL English
AB The cell surface of Bacillus stearothermophilus PV72 is covered by a regular surface layer (S-layer) composed of a single species of protein, SbsA, with a molecular weight of 130000. Recently, the sequence of the corresponding gene (sbsA) has been determined. The SbsA coding region including the signal sequence was cloned as a polymerase chain reaction (PCR) product into a low-copy-number vector under the transcriptional control of the .lambda.pL promoter. Expression of sbsA was shown to be thermally inducible from the resulting vector pBK4 in a strain of Escherichia coli expressing the .lambda.cI857 from the chromosome. As shown by ultrathin sectioning of whole cells and immunogold labelling using SbsA-specific antibodies, expression of sbsA in E. coli led to accumulation of sheet-like self-assembling products of the protein in the cytoplasm. No SbsA protein was detected either in the periplasm or in the supernatant fractions. Long-term expression of sbsA from pBK4, including in the late stationary phase, did not lead to degradation of SbsA.

L15 ANSWER 11 OF 14 CAPLUS COPYRIGHT 2000 ACS
AN 1996:492078 CAPLUS
DN 125:213353
TI Analysis of S-layer proteins and genes
AU **Kuen, Beatrix**; Lubitz, Werner
CS Austria
SO Cryst. Bact. Cell Surf. Proteins (1996), 77-102. Editor(s): Sleytr, Uwe B. Publisher: Landes, Austin, Tex.
CODEN: 63EDAO
DT Conference; General Review
LA English
AB A review with 75 refs. Surface layers (S-layers) are regularly ordered proteins found as the outermost cell envelope component of many bacteria. The authors discuss similarities and common characteristics of currently know S-layer genes.

L15 ANSWER 12 OF 14 LIFESCI COPYRIGHT 2000 CSA
AN 96:48838 LIFESCI
TI Heterologous expression and self-assembly of the **S-layer protein** SbsA of Bacillus stearothermophilus in Escherichia coli
AU **Kuen, B.**; Sara, M.; Lubitz, W.
CS Inst. Mikrobiol. und Genet., Univ. Wien Dr. Bohrg. 9, A-1030 Wien, Austria
SO MOL. MICROBIOL., (1995) vol. 19, no. 3, pp. 495-503.
ISSN: 0950-382X.
DT Journal
FS J

LA English
SL English
AB The cell surface of Bacillus stearothermophilus PV72 is covered by a regular surface layer (S-layer) composed of a single species of protein, SbsA, with a molecular weight of 130 000. Recently, the sequence of the corresponding gene (sbsA) has been determined. The SbsA coding region including the signal sequence was cloned as a polymerase chain reaction (PCR) product into a low-copy-number vector under the transcriptional control of the lambda pL promoter. Expression of sbsA was shown to be thermally inducible from the resulting vector pBK4 in a strain of Escherichia coli expressing the lambda cI857 from the chromosome. As shown by ultrathin sectioning of whole cells and immunogold labelling using SbsA-specific antibodies, expression of sbsA in E. coli led to accumulation of sheet-like self-assembling products of the protein in the cytoplasm. No SbsA protein was detected either in the periplasm or in the supernatant fractions. Long-term expression of sbsA from pBK4, including in the late stationary phase, did not lead to degradation of SbsA.

L15 ANSWER 13 OF 14 EMBASE COPYRIGHT 2000 ELSEVIER SCI. B.V.DUPLICATE 8
AN 94230051 EMBASE
DN 1994230051
TI Sequence analysis of the sbsA gene encoding the 130-kDa surface-layer protein of Bacillus stearothermophilus strain PV72.
AU Kuen B.; Sleytr U.B.; Lubitz W.
CS Inst. of Microbiology and Genetics, University of Vienna, Dr. Bohrgasse 9,A-1030 Vienna, Austria
SO Gene, (1994) 145/1 (115-120).
ISSN: 0378-1119 CODEN: GENED6
CY Netherlands
DT Journal; Article
FS 004 Microbiology
029 Clinical Biochemistry
LA English
SL English
AB Bacillus stearothermophilus (Bs) contains a surface-layer (S-layer) protein (SbsA), which forms a hexagonal array on the cell wall. In order to understand the structural/functional relationship of SbsA from Bs PV72, the entire nucleotide (nt) sequence of the sbsA gene was determined from three overlapping fragments. The 3'-end was cloned and expressed in Escherichia coli, whereas the 5'-region was amplified from the genome of Bs PV72 by the polymerase chain reaction using two overlapping fragments. The open reading frame (3684 nt) of sbsA is predicted to encode a protein of 1228 amino acids (aa). The SbsA is synthesized with a leader sequence of 30 aa. The predicted SbsA aa profile was similar to most other sequenced S-layer proteins, containing more acidic than basic aa (pI 5.1) and a very low amount of sulfur-containing aa. Based on aa sequence data, SbsA has weak homology of with the S-layer proteins from B. sphaericus, Rickettsia rickettsii, B. brevis HPD31 and B. brevis 47 (OWP).

L15 ANSWER 14 OF 14 CAPLUS COPYRIGHT 2000 ACS
AN 1995:29574 CAPLUS
DN 122:76093
TI Structural and functional analysis of the **S-layer protein** from Bacillus stearothermophilus
AU **Kuen, Beatrix**; Lubitz, Werner; Barton, Geoffrey J.
CS Institute Microbiology and Genetics, University Vienna, Vienna, Austria
SO NATO ASI Ser., Ser. A (1993), 252(ADVANCES IN BACTERIAL PARACRYSTALLINE SURFACE LAYERS), 143-9
CODEN: NALSDJ; ISSN: 0258-1213
DT Journal; General Review
LA English
AB A review and discussion with 16 refs.

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=> e truppe michaela/au

E1            4     TRUPPE MICHAEL/AU
E2            2     TRUPPE MICHAEL J/AU
E3            3 --> TRUPPE MICHAELA/AU
E4            1     TRUPPE PETER/AU
E5            1     TRUPPE ROBERT B/AU
E6            1     TRUPPE ROBERT E/AU
E7            2     TRUPPE S/AU
E8           53     TRUPPE W/AU
E9            8     TRUPPE WOLFGANG/AU
E10           3     TRUPPEL I/AU
E11           1     TRUPPEL JAIR DO AMARAL/AU
E12           1     TRUPPEL W L/AU

=> s e1-e3

L16           9 ("TRUPPE MICHAEL"/AU OR "TRUPPE MICHAEL J"/AU OR "TRUPPE
MICHAEL
                A"/AU)

=> dup rem l16

PROCESSING COMPLETED FOR L16
L17           8 DUP REM L16 (1 DUPLICATE REMOVED)

=> d ti 1-8


L17  ANSWER 1 OF 8  BIOSIS  COPYRIGHT 2000 BIOSIS
TI   Computer-aided positioning of dental implants: Preliminary results.

L17  ANSWER 2 OF 8  USPATFULL
TI     Method of imaging a person's jaw and a model therefor

L17  ANSWER 3 OF 8  USPATFULL
TI     System and method for displaying a structural data image in real-time
       correlation with moveable body

L17  ANSWER 4 OF 8  USPATFULL
TI     Apparatus and method for registration of points of a data field with
       respective points of an optical image

L17  ANSWER 5 OF 8  USPATFULL
TI     Method for displaying moveable bodies

L17  ANSWER 6 OF 8  CAPLUS  COPYRIGHT 2000 ACS
TI   Expression of S-layer proteins in Gram-negative bacteria and recombinant
     chimeric S-layer proteins for use as vaccines

L17  ANSWER 7 OF 8  BIOSIS  COPYRIGHT 2000 BIOSIS
TI   Otorhinolaryngologic computer-assisted biopsies of the Iceman.

L17  ANSWER 8 OF 8  BIOSIS  COPYRIGHT 2000 BIOSIS       DUPLICATE 1
TI   IV. Molecular biology of S-layers.


=>

=> d bib ab 6 8


L17  ANSWER 6 OF 8  CAPLUS  COPYRIGHT 2000 ACS
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AN 1997:536912 CAPLUS
DN 127:201021
TI Expression of S-layer proteins in Gram-negative bacteria and recombinant chimeric S-layer proteins for use as vaccines
IN Lubitz, Werner; Sleytr, Uwe; Kuen, Beatrix; **Truppe, Michaela**; Howorka, Stefan; Resch, Stepanka; Schroll, Gerhard; Sara, Margit
PA Lubitz, Werner, Austria; Sleytr, Uwe; Kuen, Beatrix; Truppe, Michaela; Howorka, Stefan; Resch, Stepanka; Schroll, Gerhard; Sara, Margit
SO PCT Int. Appl., 65 pp.
CODEN: PIXXD2
DT Patent
LA German
FAN.CNT 1

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
PI WO 9728263	A1	19970807	WO 1997-EP432	19970131
W: AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GE, HU, IL, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, TJ, TM, TR, TT, UA, UG, US, UZ, VN, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM				
RW: KE, LS, MW, SD, SZ, UG, AT, BE, CH, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, BF, BJ, CF, CG, CI, CM, GA, GN, ML, MR, NE, SN, TD, TG				
DE 19603649	A1	19970807	DE 1996-19603649	19960201
CA 2245584	AA	19970807	CA 1997-2245584	19970131
AU 9717203	A1	19970822	AU 1997-17203	19970131
AU 713999	B2	19991216		
EP 882129	A1	19981209	EP 1997-904360	19970131
R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, SE, MC, PT, IE, FI				
CN 1213402	A	19990407	CN 1997-192940	19970131
JP 2000503850	T2	20000404	JP 1997-527307	19970131

PRAI DE 1996-19603649 19960201
WO 1997-EP432 19970131

AB The invention concerns processess for the recombinant prepn. of S-layer proteins in Gram-neg. host cells. In addn., the nucleotide sequence of a new S-layer gene, the sbsB gene of Bacillus stearothermophilus, and a process for prepn. of modified S-layer proteins is disclosed.
Recombinant
Escherichia coli expressing the sbsA gene of B. stearothermophilus and chimeric sbsA genes encoding SbsA into which various peptides, proteins and enzymes have been inserted were prepd. and cultured to produce the proteins.

L17 ANSWER 8 OF 8 BIOSIS COPYRIGHT 2000 BIOSIS DUPLICATE 1
AN 1997:416279 BIOSIS
DN PREV199799715482
TI IV. Molecular biology of S-layers.
AU Bahl, Hubert; Scholz, Holger; Bayan, Nicolas (1); Chami, Mohamed; Leblon, Gerard; Gulik-Krzywicki, Thaddee; Shechter, Emanuel; Fouet, Agnes; Mesnage, Stephane; Tosi-Couture, Evelyne; Gounon, Pierre; Mock, Michele; De Macario, Everly Conway; Macario, Alberto J. L.; Fernandez-Herrero, Luis
A.; Olabarria, Garbine; Berenguer, Jose; Blazer, Martin J.; Kuen, Beatrix; Lubitz, Werner; Sara, Margit; Pouwels, Peter H.; Koeln, Carin P. A. M.; Boot, Hein J.; Palva, Airi; **Truppe, Michaela**; Howorka, Stephan; Schroll, Gerhard; Lechleitner, Sonja; Resch, Stephnie
CS (1) Lab. Biomembranes, URA 1116 CNRS, Univ. Paris-Sud, F-91405 Orsay France
SO FEMS Microbiology Reviews, (1997) Vol. 20, No. 1-2, pp. 47-98.
ISSN: 0168-6445.
DT General Review
LA English

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E1          14     HOWORKA S/AU
E2           1     HOWORKA SIEGFRIED/AU
E3           8 --> HOWORKA STEFAN/AU
E4           2     HOWORKA STEPHAN/AU
E5           1     HOWORKO ADOLF C/AU
E6           1     HOWORKO N/AU
E7           1     HOWORT P/AU
E8           6     HOWORTH A/AU
E9          13     HOWORTH A J/AU
E10          1     HOWORTH ALISON/AU
E11          2     HOWORTH ALISON J/AU
E12         28     HOWORTH B/AU

=> s e1 or e3 or e4

L18         24 "HOWORKA S"/AU OR "HOWORKA STEFAN"/AU OR "HOWORKA STEPHAN"/AU

=> s l18 and s layer protein

L19         13 L18 AND S LAYER PROTEIN

=> dup rem l19

PROCESSING COMPLETED FOR L19
L20          4 DUP REM L19 (9 DUPLICATES REMOVED)

=> d bib ab 1-4


L20  ANSWER 1 OF 4  EMBASE  COPYRIGHT 2000 ELSEVIER SCI. B.V.DUPLICATE 1
AN   1999088729  EMBASE
TI   Self-assembly product formation of the Bacillus stearothermophilus
PV72/p6
     S-layer protein SbsA in the course of
     autolysis of Bacillus subtilis.
AU   Howorka S.; Sara M.; Lubitz W.; Kuen B.
CS   B. Kuen, Institut Mikrobiologie and Genetik, Universitat Wien, Dr.
     Bohrgasse 9, A-1030 Vienna, Austria. oetzi@gem.univie.ac.at
SO   FEMS Microbiology Letters, (1999) 172/2 (187-196).
     Refs: 20
     ISSN: 0378-1097  CODEN: FMLED7
PUI  S 0378-1097(99)00040-3
CY   Netherlands
DT   Journal; Article
FS   004     Microbiology
LA   English
SL   English
AB   In order to achieve high level expression and to study the release of a
     protein capable of self-assembly, the gene encoding the crystalline cell
     surface (S-layer) protein SbsA of Bacillus
     stearothermophilus PV72/p6, including its signal sequence, was cloned and
     expressed in Bacillus subtilis. To obtain high level expression, a
tightly
     regulated, xylose-inducible, stably replicating multicopy-plasmid vector
     was constructed. After induction of expression, the S-
     layer protein made up about 15% of the total cellular
     protein content, which was comparable to the SbsA content of B.
     stearothermophilus PV72/p6 cells. During all growth stages, SbsA was
     poorly secreted to the ambient cellular environment by B. subtilis.
     Extraction of whole cells with guanidine hydrochloride showed that in
late
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stationary growth phase cells 65% of the synthesised SbsA was retained in the peptidoglycan-containing layer, indicating that the rigid cell wall layer was a barrier for efficient SbsA secretion. Electron microscopic investigation revealed that SbsA release from the peptidoglycan-containing layer started in the late stationary growth phase at distinct sites at the cell surface leading to the formation of extracellular self-assembly products which did not adhere to the cell wall surface. In addition, intracellular sheet-like SbsA self-assembly products which followed the curvature of the cell became visible in partly lysed cells. Intracellularly formed self-assembly products remained intact even after complete lysis of the rigid cell envelope layer. Copyright (C) 1999 Federation of European Microbiological Societies.

L20 ANSWER 2 OF 4 CAPLUS COPYRIGHT 2000 ACS
AN 1997:536912 CAPLUS
DN 127:201021
TI Expression of S-layer proteins in Gram-negative bacteria and recombinant chimeric S-layer proteins for use as vaccines
IN Lubitz, Werner; Sleytr, Uwe; Kuen, Beatrix; Truppe, Michaela; **Howorka, Stefan**; Resch, Stepanka; Schroll, Gerhard; Sara, Margit
PA Lubitz, Werner, Austria; Sleytr, Uwe; Kuen, Beatrix; Truppe, Michaela; Howorka, Stefan; Resch, Stepanka; Schroll, Gerhard; Sara, Margit
SO PCT Int. Appl., 65 pp.
CODEN: PIXXD2
DT Patent
LA German
FAN.CNT 1

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
PI WO 9728263	A1	19970807	WO 1997-EP432	19970131
W: AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GE, HU, IL, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, TJ, TM, TR, TT, UA, UG, US, UZ, VN, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM				
RW: KE, LS, MW, SD, SZ, UG, AT, BE, CH, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, BF, BJ, CF, CG, CI, CM, GA, GN, ML, MR, NE, SN, TD, TG				
DE 19603649	A1	19970807	DE 1996-19603649	19960201
CA 2245584	AA	19970807	CA 1997-2245584	19970131
AU 9717203	A1	19970822	AU 1997-17203	19970131
AU 713999	B2	19991216		
EP 882129	A1	19981209	EP 1997-904360	19970131
R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, SE, MC, PT, IE, FI				
CN 1213402	A	19990407	CN 1997-192940	19970131
JP 2000503850	T2	20000404	JP 1997-527307	19970131
PRAI DE 1996-19603649	19960201			
WO 1997-EP432	19970131			

AB The invention concerns processess for the recombinant prepn. of S-layer proteins in Gram-neg. host cells. In addn., the nucleotide sequence of a new S-layer gene, the sbsB gene of Bacillus stearothermophilus, and a process for prepn. of modified S-layer proteins is disclosed. Recombinant Escherichia coli expressing the sbsA gene of B. stearothermophilus and chimeric sbsA genes encoding SbsA into which various peptides, proteins and enzymes have been inserted were prepd. and cultured to produce the proteins.

L20 ANSWER 3 OF 4 EMBASE COPYRIGHT 2000 ELSEVIER SCI. B.V.DUPLICATE 2
AN 97267948 EMBASE
DN 1997267948
TI IV. Molecular biology of S-layers.

AU Bahl H.; Scholz H.; Bayan N.; Chami M.; Leblon G.; Gulik-Krzywicki T.; Shechter E.; Fouet A.; Mesnage S.; Tosi-Couture E.; Gounon P.; Mock M.; De
Macario E.C.; Macario A.J.L.; Fernandez-Herrero L.A.; Olabarria G.; Berenguer J.; Blaser M.J.; Kuen B.; Lubitz W.; Sara M.; Pouwels P.H.; Kolen C.P.A.M.; Boot H.J.; Palva A.; Truppe M.; **Howorka S.**; Schroll G.; Lechleitner S.; Resch S.

CS Dr. N. Bayan, Laboratoire des Biomembranes, URA 1116 CNRS, Universite de Paris-Sud, F-91405 Orsay, France

SO FEMS Microbiology Reviews, (1997) 20/1-2 (47-98).
Refs: 197
ISSN: 0168-6445 CODEN: FMREE4

PUI S 0168-6445(97)00050-8

CY Netherlands

DT Journal; General Review

FS 004 Microbiology

LA English

SL English

AB In this chapter we report on the molecular biology of crystalline surface layers of different bacterial groups. The limited information indicates that there are many variations on a common theme. Sequence variety, antigenic diversity, gene expression, rearrangements, influence of environmental factors and applied aspects are addressed. There is considerable variety in the S-layer composition, which was elucidated by sequence analysis of the corresponding genes. In Corynebacterium glutamicum one major cell wall protein is responsible for the formation of a highly ordered, hexagonal array. In contrast, two abundant surface proteins form the S-layer of Bacillus anthracis. Each protein possesses three S-layer homology motifs and one protein could be a virulence factor.
The antigenic diversity and ABC transporters are important features, which have been studied in methanogenic archaea. The expression of the S-layer components is controlled by three genes in the case of Thermus thermophilus. One has repressor activity on the S-layer gene promoter, the second codes for the **S-layer protein**. The rearrangement by reciprocal recombination was investigated in Campylobacter fetus. 7-8 S-layer proteins with a high degree of homology at the 5' and 3' ends were found. Environmental changes influence the surface properties of Bacillus stearothermophilus. Depending on oxygen supply, this species produces different S-layer proteins. Finaly, the molecular bases for some applications are discussed. Recombinant S-layer fusion proteins have been designed for biotechnology.

L20 ANSWER 4 OF 4 EMBASE COPYRIGHT 2000 ELSEVIER SCI. B.V.DUPLICATE 3

AN 96346348 EMBASE

DN 1996346348

TI 2-D protein crystals as an immobilization matrix for producing reaction zones in dipstick-style immunoassays.

AU Breitwieser A.; Kupcu S.; **Howorka S.**; Weiger S.; Langer C.; Hoffmann- Sommergruber K.; Scheiner O.; Sleytr U.B.; Sara M.

CS ZULB, Inst. fur Molekulare Nanotechnologie, Universitat fur Bodenkultur, Gregor Mendelstrasse 33,A-1180 Vienna, Austria

SO BioTechniques, (1996) 21/5 (918-925).
ISSN: 0736-6205 CODEN: BTNQDO

CY United States

DT Journal; Article

FS 004 Microbiology
027 Biophysics, Bioengineering and Medical Instrumentation
029 Clinical Biochemistry

LA English

SL English

AB In the present study, the applicability of crystalline bacterial cell-

surface layers (S-layers) as novel immobilization matrices and reaction zones for dipstick-style immunoassays was investigated. For this purpose, S-layer- carrying cell-wall fragments from Bacillus sphaericus CCM 2120 were deposited on a microporous support, and the **S-layer protein** was cross-linked with glutaraldehyde. For developing appropriate test systems, either human IgG was directly linked to the carboxylic acid groups from the **S-layer protein** or it was immobilized using Protein A or, after biotinylation, using streptavidin. A clear correlation was obtained between the amount of anti-human IgG applied and the absorbance values in the immunoassays. S-layers with covalently bound recombinant major birch pollen allergen were used for quantitative and semiquantitative determination of an antibody raised against it. Using S- layers as an immobilization matrix in comparison to amorphous polymers has advantages in that the closed monolayers of functional macromolecules on their outermost surface allows for strong signals in immunoassays, almost completely eliminates background and prevents diffusion.

```
=> e resch stephanka/au

E1            1     RESCH STEPANKA/AU
E2            4     RESCH STEPHANIE/AU
E3            0 --> RESCH STEPHANKA/AU
E4            1     RESCH STEPHNIE/AU
E5            1     RESCH STEVEN C/AU
E6            3     RESCH SYLVIA/AU
E7           34     RESCH T/AU
E8            1     RESCH T A/AU
E9            1     RESCH T J/AU
E10           1     RESCH T L/AU
E11           1     RESCH TH/AU
E12           2     RESCH THOMAS/AU

=> s e1 or e2 or e4

L21           6 "RESCH STEPANKA"/AU OR "RESCH STEPHANIE"/AU OR "RESCH
STEPHNIE"/
                AU

=> d ti 1-6


L21  ANSWER 1 OF 6  BIOSIS  COPYRIGHT 2000 BIOSIS
TI   Heterologous PHIX174 gene E-expression in Ralstonia eutropha: E-mediated
     lysis is not restricted to gamma-subclass of proteobacteria.

L21  ANSWER 2 OF 6  BIOSIS  COPYRIGHT 2000 BIOSIS
TI   IV. Molecular biology of S-layers.

L21  ANSWER 3 OF 6  CAPLUS  COPYRIGHT 2000 ACS
TI   Production of genetically engineered S-layer protein that is secreted
into
     the periplasm or extracellularly and that can contain integrated proteins
     for affinity and immuno reactions

L21  ANSWER 4 OF 6  CAPLUS  COPYRIGHT 2000 ACS
TI   Heterologous .PHI.X174 gene E-expression in Ralstonia eutropha:
E-mediated
     lysis is not restricted to .gamma.-subclass of proteobacteria

L21  ANSWER 5 OF 6  CAPLUS  COPYRIGHT 2000 ACS
TI   Expression of S-layer proteins in Gram-negative bacteria and recombinant
     chimeric S-layer proteins for use as vaccines
```

L21 ANSWER 6 OF 6 CAPLUS COPYRIGHT 2000 ACS
TI IV. Molecular biology of S-layers

=> dup rem l21

PROCESSING COMPLETED FOR L21
L22 4 DUP REM L21 (2 DUPLICATES REMOVED)

=> d bib ab 1-4

L22 ANSWER 1 OF 4 CAPLUS COPYRIGHT 2000 ACS
AN 1999:96508 CAPLUS
DN 130:178339
TI Production of genetically engineered S-layer protein that is secreted into the periplasm or extracellularly and that can contain integrated proteins for affinity and immuno reactions
IN Lubitz, Werner; **Resch, Stephanie**
PA Austria
SO Ger. Offen., 34 pp.
CODEN: GWXXBX
DT Patent
LA German
FAN.CNT 1

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
PI DE 19732829	A1	19990204	DE 1997-19732829	19970730
WO 9906567	A1	19990211	WO 1998-EP4723	19980727
W: AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GE, GH, GM, HR, HU, ID, IL, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, UA, UG, US, UZ, VN, YU, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM				
RW: GH, GM, KE, LS, MW, SD, SZ, UG, ZW, AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG				
AU 9890705	A1	19990222	AU 1998-90705	19980727
EP 1005553	A1	20000607	EP 1998-942648	19980727
R: AT, BE, CH, DE, DK, ES, FR, GB, IT, LI, NL, SE, IE				

PRAI DE 1997-19732829 19970730
WO 1998-EP4723 19980727
AB The invention concerns the prodn. of recombinant S-layer protein expressed in Gram-neg. prokaryote or eukaryote host cells using the sbsA and sbsB clones of the Bacillus stearothermophilus PV72, that code for the S-layer protein and the prokaryote signal peptide; the vector also contains inserts at convenient sites that code for various peptides, e.g. cysteine residues, DNA-binding epitopes, metal-binding epitopes, allergens, antigens, streptavidin, enzymes etc. In case the fusion protein is expressed in eukaryotes, the vector includes sequences coding for eukaryote signal peptides. The host cell contains at least two types of genes that code for the a non-modified S-layer protein and for a modified S-layer protein that is fused with a peptide used biochem. reactions. E.coli is a typical host cell.

L22 ANSWER 2 OF 4 BIOSIS COPYRIGHT 2000 BIOSIS DUPLICATE 1
AN 1999:58090 BIOSIS
DN PREV199900058090
TI Heterologous PHIX174 gene E-expression in Ralstonia eutropha: E-mediated lysis is not restricted to gamma-subclass of proteobacteria.
AU Schroll, Gerhard (1); **Resch, Stephanie**; Gruber, Karin; Wanner, Gerhard; Lubitz, Werner
CS (1) Inst. Microbiol. Genet., Univ. Vienna, Dr. Bohr-Gasse 9, A-1030 Vienna

Austria
SO Journal of Biotechnology, (Dec. 11, 1998) Vol. 66, No. 2-3, pp. 211-217. ISSN: 0168-1656.
DT Article
LA English
AB E-lysis of Ralstonia eutropha H16, which belongs to the beta-subclass, was undertaken to verify whether transmembrane tunnel formation is possible in bacteria which do not belong to the enterobacteriaceae. For this purpose, a new gene E expression plasmid, pKG12, with two origins of replication, oriV and oriT, from plasmid pRP4, chloramphenicol and kanamycin resistance genes and a casette composed of lambdacI857 and lambdapR gene E was constructed. Temperature upshift of R. eutropha H16 (pKG12) from 28 to 45degreeC during exponential growth resulted in lysis of the strain with features characteristic of E-mediated lysis of Escherichia coli. The cytoplasmic contents released can easily be separated from the still intact envelope fraction by centrifugation or filtration. As R. eutropha H16 represents an important industrial organism, E-mediated lysis could facilitate procedures for the recovery of intracellular mediators or products like polyhydroxyalkanoates.

L22 ANSWER 3 OF 4 CAPLUS COPYRIGHT 2000 ACS
AN 1997:536912 CAPLUS
DN 127:201021
TI Expression of S-layer proteins in Gram-negative bacteria and recombinant chimeric S-layer proteins for use as vaccines
IN Lubitz, Werner; Sleytr, Uwe; Kuen, Beatrix; Truppe, Michaela; Howorka, Stefan; **Resch, Stepanka**; Schroll, Gerhard; Sara, Margit
PA Lubitz, Werner, Austria; Sleytr, Uwe; Kuen, Beatrix; Truppe, Michaela; Howorka, Stefan; Resch, Stepanka; Schroll, Gerhard; Sara, Margit
SO PCT Int. Appl., 65 pp.
CODEN: PIXXD2
DT Patent
LA German
FAN.CNT 1

	PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
PI	WO 9728263	A1	19970807	WO 1997-EP432	19970131
	W: AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GE, HU, IL, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, TJ, TM, TR, TT, UA, UG, US, UZ, VN, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM				
	RW: KE, LS, MW, SD, SZ, UG, AT, BE, CH, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, BF, BJ, CF, CG, CI, CM, GA, GN, ML, MR, NE, SN, TD, TG				
	DE 19603649	A1	19970807	DE 1996-19603649	19960201
	CA 2245584	AA	19970807	CA 1997-2245584	19970131
	AU 9717203	A1	19970822	AU 1997-17203	19970131
	AU 713999	B2	19991216		
	EP 882129	A1	19981209	EP 1997-904360	19970131
	R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, SE, MC, PT, IE, FI				
	CN 1213402	A	19990407	CN 1997-192940	19970131
	JP 2000503850	T2	20000404	JP 1997-527307	19970131
PRAI	DE 1996-19603649	19960201			
	WO 1997-EP432	19970131			

AB The invention concerns processess for the recombinant prepn. of S-layer proteins in Gram-neg. host cells. In addn., the nucleotide sequence of a new S-layer gene, the sbsB gene of Bacillus stearothermophilus, and a process for prepn. of modified S-layer proteins is disclosed.
Recombinant Escherichia coli expressing the sbsA gene of B. stearothermophilus and

chimeric sbsA genes encoding SbsA into which various peptides, proteins and enzymes have been inserted were prepd. and cultured to produce the proteins.

L22 ANSWER 4 OF 4 BIOSIS COPYRIGHT 2000 BIOSIS DUPLICATE 2
AN 1997:416279 BIOSIS
DN PREV199799715482
TI IV. Molecular biology of S-layers.
AU Bahl, Hubert; Scholz, Holger; Bayan, Nicolas (1); Chami, Mohamed; Leblon, Gerard; Gulik-Krzywicki, Thaddee; Shechter, Emanuel; Fouet, Agnes; Mesnage, Stephane; Tosi-Couture, Evelyne; Gounon, Pierre; Mock, Michele; De Macario, Everly Conway; Macario, Alberto J. L.; Fernandez-Herrero, Luis A.; Olabarria, Garbine; Berenguer, Jose; Blazer, Martin J.; Kuen, Beatrix; Lubitz, Werner; Sara, Margit; Pouwels, Peter H.; Koeln, Carin P. A. M.; Boot, Hein J.; Palva, Airi; Truppe, Michaela; Howorka, Stephan; Schroll, Gerhard; Lechleitner, Sonja; **Resch, Stephnie**
CS (1) Lab. Biomembranes, URA 1116 CNRS, Univ. Paris-Sud, F-91405 Orsay France
SO FEMS Microbiology Reviews, (1997) Vol. 20, No. 1-2, pp. 47-98. ISSN: 0168-6445.
DT General Review
LA English

=> e schroll gerhard/au

E1	6		SCHROLL GENE E/AU
E2	2		SCHROLL GEORG/AU
E3	8	-->	SCHROLL GERHARD/AU
E4	2		SCHROLL GOTTFRIED/AU
E5	4		SCHROLL GUENTER/AU
E6	43		SCHROLL GUSTAV/AU
E7	27		SCHROLL H/AU
E8	1		SCHROLL HANS/AU
E9	2		SCHROLL HARSTED BJARNE/AU
E10	2		SCHROLL HENNING/AU
E11	4		SCHROLL J/AU
E12	1		SCHROLL J T/AU

=> s e3

L23 8 "SCHROLL GERHARD"/AU

=> dup rem l23

PROCESSING COMPLETED FOR L23
L24 5 DUP REM L23 (3 DUPLICATES REMOVED)

=> d ti 1-5

L24 ANSWER 1 OF 5 CAPLUS COPYRIGHT 2000 ACS
TI Conversion of solvent evaporation residues from the AB- (acetone-butanol) bioprocess into bacterial cells accumulating thermoplastic polyesters

L24 ANSWER 2 OF 5 BIOSIS COPYRIGHT 2000 BIOSIS DUPLICATE 1
TI Heterologous PHIX174 gene E-expression in Ralstonia eutropha: E-mediated lysis is not restricted to gamma-subclass of proteobacteria.

L24 ANSWER 3 OF 5 CAPLUS COPYRIGHT 2000 ACS
TI Expression of S-layer proteins in Gram-negative bacteria and recombinant chimeric S-layer proteins for use as vaccines

L24 ANSWER 4 OF 5 BIOSIS COPYRIGHT 2000 BIOSIS DUPLICATE 2
TI IV. Molecular biology of S-layers.

L24 ANSWER 5 OF 5 BIOSIS COPYRIGHT 2000 BIOSIS DUPLICATE 3
TI Characterization of a new Pseudomonas isolate, capable of accumulating polyesters of medium chain length 3-hydroxyalkanoic acids.

=> d bib ab 1-5

L24 ANSWER 1 OF 5 CAPLUS COPYRIGHT 2000 ACS
AN 2000:226785 CAPLUS
DN 132:307309
TI Conversion of solvent evaporation residues from the AB- (acetone-butanol) bioprocess into bacterial cells accumulating thermoplastic polyesters
AU Parrer, Gunter; **Schroll, Gerhard**; Gapes, J. Richard; Lubitz, Werner; Schuster, K. Christian
CS Institute for Microbiology and Genetics, University of Vienna, Vienna, A-1030, Austria
SO J. Mol. Microbiol. Biotechnol. (2000), 2(1), 81-86
CODEN: JMMBFF; ISSN: 1464-1801
PB Horizon Scientific Press
DT Journal
LA English
AB In a bioconversion study based on utilization of byproducts from the AB- (acetone-butanol) bioprocess a new isolated Gram-neg. solvent tolerant bacterium was used to convert the AB process residue after removal of the major part of the solvents. The bacterium identified as a representative of the genus Alcaligenes (designated as Alcaligenes sp. G) was capable of growth up to optical densities ranging from 8 to 20 and simultaneously of polyhydroxyalkanoate-(PHA-)accumulation up to 40% per dry wt. A standardised medium based on AB byproducts contg. 7 g/l of butyrate and 5 g/l of acetate at pH 7.5 was used in our studies for bioconversion into PHAs. Concns. of 1-butanol, which is known for its membrane damaging properties in micro-organisms, were tolerated in the AB byproducts medium up to 4 g/l without significant inhibition of cellular growth. No inhibition of growth was obsd., when the medium was adjusted to 40 g/l butyrate. Due to the toxicity of the remaining 1-butanol maintenance of sterility is of no high priority during the process. The use of acetate and butyrate from an AB process is expected to provide a higher return-on-investment than the combustion of biogas to help meet energy demands.
RE.CNT 37
RE
(5) Byrom, D; Novel biodegradabel microbial biopolymers 1990, P113 CAPLUS
(6) Choi, J; Appl Environ Microbiol 1998, V64, P4897 CAPLUS
(7) Choi, J; Appl Microbiol Biotechnol 1999, V51, P13 CAPLUS
(8) Fernandez-Castillo, R; Appl Environm Microbiol 1986, V51, P214 CAPLUS
(10) Gapes, J; Appl Environ Microbiol 1996, V62, P3210 CAPLUS
ALL CITATIONS AVAILABLE IN THE RE FORMAT

L24 ANSWER 2 OF 5 BIOSIS COPYRIGHT 2000 BIOSIS DUPLICATE 1
AN 1999:58090 BIOSIS
DN PREV199900058090
TI Heterologous PHIX174 gene E-expression in Ralstonia eutropha: E-mediated lysis is not restricted to gamma-subclass of proteobacteria.
AU **Schroll, Gerhard (1)**; Resch, Stephanie; Gruber, Karin; Wanner, Gerhard; Lubitz, Werner
CS (1) Inst. Microbiol. Genet., Univ. Vienna, Dr. Bohr-Gasse 9, A-1030 Vienna
Austria
SO Journal of Biotechnology, (Dec. 11, 1998) Vol. 66, No. 2-3, pp. 211-217.
ISSN: 0168-1656.
DT Article

LA English
AB E-lysis of Ralstonia eutropha H16, which belongs to the beta-subclass, was undertaken to verify whether transmembrane tunnel formation is possible in bacteria which do not belong to the enterobacteriaceae. For this purpose, a new gene E expression plasmid, pKG12, with two origins of replication, oriV and oriT, from plasmid pRP4, chloramphenicol and kanamycin resistance genes and a casette composed of lambdacI857 and lambdapR gene E was constructed. Temperature upshift of R. eutropha H16 (pKG12) from 28 to 45degreeC during exponential growth resulted in lysis of the strain with features characteristic of E-mediated lysis of Escherichia coli. The cytoplasmic contents released can easily be separated from the still intact envelope fraction by centrifugation or filtration. As R. eutropha H16 represents an important industrial organism, E-mediated lysis could facilitate procedures for the recovery of intracellular mediators or products like polyhydroxyalkanoates.

L24 ANSWER 3 OF 5 CAPLUS COPYRIGHT 2000 ACS
AN 1997:536912 CAPLUS
DN 127:201021
TI Expression of S-layer proteins in Gram-negative bacteria and recombinant chimeric S-layer proteins for use as vaccines
IN Lubitz, Werner; Sleytr, Uwe; Kuen, Beatrix; Truppe, Michaela; Howorka, Stefan; Resch, Stepanka; **Schroll, Gerhard**; Sara, Margit
PA Lubitz, Werner, Austria; Sleytr, Uwe; Kuen, Beatrix; Truppe, Michaela; Howorka, Stefan; Resch, Stepanka; Schroll, Gerhard; Sara, Margit
SO PCT Int. Appl., 65 pp.
CODEN: PIXXD2
DT Patent
LA German
FAN.CNT 1

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
PI WO 9728263	A1	19970807	WO 1997-EP432	19970131
W: AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GE, HU, IL, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, TJ, TM, TR, TT, UA, UG, US, UZ, VN, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM				
RW: KE, LS, MW, SD, SZ, UG, AT, BE, CH, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, BF, BJ, CF, CG, CI, CM, GA, GN, ML, MR, NE, SN, TD, TG				
DE 19603649	A1	19970807	DE 1996-19603649	19960201
CA 2245584	AA	19970807	CA 1997-2245584	19970131
AU 9717203	A1	19970822	AU 1997-17203	19970131
AU 713999	B2	19991216		
EP 882129	A1	19981209	EP 1997-904360	19970131
R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, SE, MC, PT, IE, FI				
CN 1213402	A	19990407	CN 1997-192940	19970131
JP 2000503850	T2	20000404	JP 1997-527307	19970131

PRAI DE 1996-19603649 19960201
WO 1997-EP432 19970131

AB The invention concerns processess for the recombinant prepn. of S-layer proteins in Gram-neg. host cells. In addn., the nucleotide sequence of a new S-layer gene, the sbsB gene of Bacillus stearothermophilus, and a process for prepn. of modified S-layer proteins is disclosed.
Recombinant Escherichia coli expressing the sbsA gene of B. stearothermophilus and chimeric sbsA genes encoding SbsA into which various peptides, proteins and enzymes have been inserted were prepd. and cultured to produce the proteins.

L24 ANSWER 4 OF 5 BIOSIS COPYRIGHT 2000 BIOSIS DUPLICATE 2
AN 1997:416279 BIOSIS
DN PREV199799715482
TI IV. Molecular biology of S-layers.
AU Bahl, Hubert; Scholz, Holger; Bayan, Nicolas (1); Chami, Mohamed; Leblon, Gerard; Gulik-Krzywicki, Thaddee; Shechter, Emanuel; Fouet, Agnes; Mesnage, Stephane; Tosi-Couture, Evelyne; Gounon, Pierre; Mock, Michele; De Macario, Everly Conway; Macario, Alberto J. L.; Fernandez-Herrero, Luis
A.; Olabarria, Garbine; Berenguer, Jose; Blazer, Martin J.; Kuen, Beatrix;
Lubitz, Werner; Sara, Margit; Pouwels, Peter H.; Koeln, Carin P. A. M.; Boot, Hein J.; Palva, Airi; Truppe, Michaela; Howorka, Stephan; **Schroll, Gerhard**; Lechleitner, Sonja; Resch, Stephnie
CS (1) Lab. Biomembranes, URA 1116 CNRS, Univ. Paris-Sud, F-91405 Orsay France
SO FEMS Microbiology Reviews, (1997) Vol. 20, No. 1-2, pp. 47-98.
ISSN: 0168-6445.
DT General Review
LA English

L24 ANSWER 5 OF 5 BIOSIS COPYRIGHT 2000 BIOSIS DUPLICATE 3
AN 1996:427188 BIOSIS
DN PREV199699158244
TI Characterization of a new Pseudomonas isolate, capable of accumulating polyesters of medium chain length 3-hydroxyalkanoic acids.
AU **Schroll, Gerhard**; Denner, Ewald B. M.; Roelleke, Sabine; Lubitz, Werner; Busse, Hans-Juergen (1)
CS (1) Inst. for Microbiol. Genetics, Univ. Vienna, Dr. Bohr-Gasse 9, A-1030 Vienna Austria
SO Journal of Biotechnology, (1996) Vol. 47, No. 1, pp. 53-63.
ISSN: 0168-1656.
DT Article
LA English
AB In a screening program for bacteria) strains which produce interesting compositions of polyhydroxyalkanoates (PHAs) the strain PHA1 was isolated displaying an unusual PHA pattern. The presence of ubiquinone with nine isoprenoid units in the side chain (Q-9), putrescine and spermidine as the
dominating compounds in the polyamine pattern, the characteristic fatty acid profile as well as partial 16S rDNA analysis clearly indicated, that the isolate is a member of the genus Pseudomonas and closely related to Pseudomonas fluorescens. The strain PHA1 and Pseudomonas fluorescens bv. IV DSM 50415 were investigated for PHA accumulation capabilities. In both strains, alkanoates which corresponded to the carbon chain length (C6-C10)
of the fatty acids added as substrate dominated in the PHA patterns and the carbon chain length was reduced by two C-atoms when undecanoate or dodecanoate was fed. Most of the other polymers produced consisted mainly of decanoate or dodecanoate. When the complex medium was supplemented with
butyric acid the incorporation of 3-OH butyric acid into the polymer was observed. This resulted in the production of PHA blends or possibly even in the production of mixed copolymers with a significant amount of 3-OH butyric acid in addition to medium chain length PHAs.

=> e sara margit/au

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E1          5     SARA M J/AU
E2          2     SARA M N/AU
E3        119 --> SARA MARGIT/AU
E4          3     SARA MARTIN N/AU
E5          8     SARA MAURIZIO/AU
E6          1     SARA MEZA C R/AU
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E9           1     SARA N/AU
E10          1     SARA O N/AU
E11          1     SARA O NURI/AU
E12          2     SARA ONGAY M J/AU

=> s e3

L25        119 "SARA MARGIT"/AU

=> s l25 and s layer protein

L26         70 L25 AND S LAYER PROTEIN

=> s l26 and fusion protein

L27          0 L26 AND FUSION PROTEIN

=> s s layer protein

   8 FILES SEARCHED...
L28        916 S LAYER PROTEIN

=> s surface layer protein

L29        345 SURFACE LAYER PROTEIN

=> s l29 and (fusion protein or heterologous)

L30         24 L29 AND (FUSION PROTEIN OR HETEROLOGOUS)

=> dup rem l30

PROCESSING COMPLETED FOR L30
L31          16 DUP REM L30 (8 DUPLICATES REMOVED)

=> d bib ab 1-16


L31   ANSWER 1 OF 16  BIOTECHDS COPYRIGHT 2000 DERWENT INFORMATION LTD
AN    2000-14903  BIOTECHDS
TI    Caulobacter host cell for expression and secretion of a
    heterologous polypeptide, useful for treating sewage, waste-water
      and in the pulping process;
         vector-mediated surface layer protein
         gene transfer, expression in host cell and recombinant protein
         production for waste-water treatment and fish vaccine
AU    Smit J
PA    Univ.British-Columbia
LO    Vancouver, British Columbia, Canada.
PI    WO 2000049163 24 Aug 2000
AI    WO 2000-CA173 21 Feb 2000
PRAI  CA 990261186 19 Feb 1999
DT    Patent
LA    English
OS    WPI: 2000-571987 [53]
AB    A Caulobacter sp. host cell for expression and secretion of a
    heterologous protein, is claimed.  Also bacterium has at least 1
      surface layer transport protein having a protein sequence homologous to
      the RasD and RasE sequence (specified).  The host also has a DNA vector
      construct encoding a protein heterologous to a surface
    layer protein of the cell and operably linked to a DNA
      encoding a Caulobacter sp. surface layer
    protein secretion signal, with the proviso that when the cell has
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transport proteins with the sequence as both the RasD and RasE proteins. Also claimed are: a method for identifying a Caulobacter sp. suitable for use as a host cell for expression and secretion of a **heterologous** protein; and a DNA construct with 1 or more restriction sites for facilitating insertion of DNA into the construct (where the construct further has DNA encoding Caulobacter sp. **surface layer protein** secretion signal not present in Caulobacter crescentus). The host cell is used for the expression and secretion of a **heterologous** protein. The modified Caulobacter sp. cells may be used to treat sewage and waste-water. They can also be used as fish vaccines. (46pp)

L31 ANSWER 2 OF 16 CAPLUS COPYRIGHT 2000 ACS
AN 2000:592847 CAPLUS
DN 133:188882
TI Requirements for protein secretion by freshwater Caulobacter and the development of the bacterium as a secretory expression host
IN Smit, John
PA University of British Columbia, Can.
SO PCT Int. Appl., 46 pp.
CODEN: PIXXD2
DT Patent
LA English
FAN.CNT 1

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
WO 2000049163	A1	20000824	WO 2000-CA173	20000221

W: AE, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CR, CU, CZ, DE, DK, DM, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM
RW: GH, GM, KE, LS, MW, SD, SL, SZ, TZ, UG, ZW, AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG

PRAI CA 1999-2261186 19990219
AB A method is provided for screening Caulobacter suitable for use as host organisms for secretion of **heterologous** polypeptides. Such have a transport protein homologous to one of the type I transport proteins known in Caulobacter crescentus. DNA constructs are also provided which code for a chimeric protein of which the C-terminus is a secretion signal of a caulobacter **surface layer protein**, other than from C. crescentus. Bacterial cells contg., or which express such DNA constructs and which may secrete the resulting protein, are also provided..
RE.CNT 3
RE
(1) Awram, P; JOURNAL OF BACTERIOLOGY 1998, V180(12), P3062 CAPLUS
(2) Univ British Columbia; WO 9734000 A 1997 CAPLUS
(3) Walker, S; J BACTERIOL 1992, V174(6), P1783 CAPLUS

L31 ANSWER 3 OF 16 MEDLINE DUPLICATE 1
AN 2000170659 MEDLINE
DN 20170659
TI S-layer gene sbsC of Bacillus stearothermophilus ATCC 12980: molecular characterization and **heterologous** expression in Escherichia coli.
AU Jarosch M; Egelseer E M; Mattanovich D; Sleytr U B; Sara M
CS Zentrum fur Ultrastrukturforschung und Ludwig Boltzmann-Insitut fur Molekulare Nanotechnologie, Universitat fur Bodenkultur, Vienna, Austria.
SO MICROBIOLOGY, (2000 Feb) 146 (Pt 2) 273-81.
Journal code: BXW. ISSN: 1350-0872.

CY ENGLAND: United Kingdom
DT Journal; Article; (JOURNAL ARTICLE)
LA English
FS Priority Journals
OS GENBANK-AF055578
EM 200007
EW 20000701
AB The cell surface of Bacillus stearothermophilus ATCC 12980 is completely covered with an oblique S-layer lattice. To investigate sequence identities and a common structure-function relationship in S-layer proteins of different B. stearothermophilus wild-type strains, the nucleotide sequence encoding the S-layer protein SbsC of B. stearothermophilus ATCC 12980 was determined by PCR techniques. The entire sbsC sequence showed an ORF of 3297 bp predicted to encode a protein of 1099 aa with a theoretical molecular mass of 115409 Da and an isoelectric point of 5.73. Primer extension analysis suggested the existence of two promoter regions. Amino acid sequence comparison between SbsC and SbsA, a previously characterized S-layer protein of B. stearothermophilus PV72/p6 which assembles into a hexagonally ordered lattice, revealed an identical secretion signal peptide, 85% identity for the N-terminal regions (aa 31-270) which do not carry any S-layer homologous motifs, but only 21% identity for the rest of the sequences. Affinity studies demonstrated that the N-terminal part of SbsC is necessary for recognition of a secondary cell wall polymer. This was in accordance with results obtained in a previous study for SbsA, thus confirming a common functional principle for the N-terminal parts of both S-layer proteins. The sbsC coding region cloned into the pET3a vector without its own upstream region, the signal sequence and the 3' transcriptional terminator led to stable expression in Escherichia coli.

L31 ANSWER 4 OF 16 USPATFULL
AN 1999:137013 USPATFULL
TI Expression and secretion of **heterologous** polypeptides from caulobacter
IN Smit, John, Richmond, Canada
Bingle, Wade H., Vancouver, Canada
Nomellini, John F., Richmond, Canada
PA The University of British Columbia, Canada (non-U.S. corporation)
PI US 5976864 19991102
AI US 1996-614377 19960312 (8)
RLI Continuation-in-part of Ser. No. US 1994-194290, filed on 9 Feb 1994, now patented, Pat. No. US 5500353 which is a continuation-in-part of Ser. No. US 1992-895367, filed on 9 Jun 1992, now abandoned
DT Utility
EXNAM Primary Examiner: Wax, Robert A.; Assistant Examiner: Nashed, Nashaat T.
LREP Fish & Richardson P.C.
CLMN Number of Claims: 14
ECL Exemplary Claim: 2
DRWN 14 Drawing Figure(s); 13 Drawing Page(s)
LN.CNT 1609
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
AB DNA constructs are provided which code for at least the extreme C-terminal amino acids of the rsaA protein of Caulobacter crescentus fused with **heterologous** polypeptides. Baterial cells containing, or which express the DNA constructs and secrete the resulting protein are also provided. Chimeric proteins including the C-terminal amino acids of the rsaA protein are provided, including chimeric proteins comprising antigenic epitopes of the Infectious Hematopoietic Necrosis Virus.

L31 ANSWER 5 OF 16 USPATFULL
AN 1999:43376 USPATFULL
TI Identification of polycystic kidney disease gene, diagnostics and treatment
IN Reeders, Stephen, Newtonville, MA, United States
Schneider, Michael, Boston, MA, United States
Glucksmann, Maria Alexandra, Somerville, MA, United States
PA Brigham and Women's Hospital, Boston, MA, United States (U.S. corporation)
Millenium Pharmaceuticals, Cambridge, MA, United States (U.S. corporation)
PI US 5891628 19990406
AI US 1995-460751 19950602 (8)
RLI Division of Ser. No. US 1995-413580, filed on 30 Mar 1995 which is a continuation-in-part of Ser. No. US 1994-253524, filed on 3 Jun 1994, now abandoned
DT Utility
EXNAM Primary Examiner: Huff, Sheela
LREP Pennie & Edmonds, LLP
CLMN Number of Claims: 30
ECL Exemplary Claim: 1
DRWN 28 Drawing Figure(s); 28 Drawing Page(s)
LN.CNT 4191
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
AB The present invention relates to the identification of the autosomal dominant polycystic kidney disease (PKD) gene and high throughput assays
to identify compounds that interfere with PKD activity. Interfering compounds that inhibit the expression, synthesis and/or bioactivity of the PKD gene product can be used therapeutically to treat polycystic kidney disease.

L31 ANSWER 6 DF 16 USPATFULL
AN 1999:24489 USPATFULL
TI Expression of surface layer proteins
IN Deblaere, Rolf Y., Waarschoot, Belgium
Desomer, Jan, Drongen, Belgium
Dhaese, Patrick, Drongen, Belgium
PA Solvay (Societe Anonyme), Brussels, Belgium (non-U.S. corporation)
PI US 5874267 19990223
WO 9519371 19950720
AI US 1996-682517 19960917 (8)
WO 1995-EP147 19950113
19960917 PCT 371 date
19960917 PCT 102(e) date
PRAI GB 1994-650 19940114
DT Utility
EXNAM Primary Examiner: Hutzell, Paula K.; Assistant Examiner: Bakalyar, Heather A.
LREP McDermott, Will & Emery
CLMN Number of Claims: 1
ECL Exemplary Claim: 1
DRWN 41 Drawing Figure(s); 37 Drawing Page(s)
LN.CNT 2742
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
AB A host cell which is provided with a S-layer comprising a fusion polypeptide consisting essentially of:

(a) at least sufficient of a S-layer protein for a S-layer composed thereof to assemble, and

(b) a **heterologous** polypeptide which is fused to either the carboxy terminus of (a) or the amino terminus of (a) and which is thereby presented on the outer surface of the said cell; can be used as a vaccine, for screening for proteins and antigens and as a support for

immobilizing an enzyme, peptide or antigen. A process of transforming B.
Sphaericus cells comprising electroporation is also provided.

L31 ANSWER 7 OF 16 MEDLINE DUPLICATE 2
AN 1999177548 MEDLINE
DN 99177548
TI The expression signals of the Lactobacillus brevis slpA gene direct efficient **heterologous** protein production in lactic acid bacteria.
AU Kahala M; Palva A
CS Agricultural Research Centre of Finland, Food Research Institute, Finland.
SO APPLIED MICROBIOLOGY AND BIOTECHNOLOGY, (1999 Jan) 51 (1) 71-8. Journal code: AMC. ISSN: 0175-7598.
CY GERMANY: Germany, Federal Republic of
DT Journal; Article; (JOURNAL ARTICLE)
LA English
FS Priority Journals
EM 199906
AB A cassette based on the expression signals of the Lactobacillus brevis surface (S)-layer protein gene (slpA) was constructed. The low-copy-number vector pKTH2095, derived from pGK12, was used as the cloning vector. The efficiency of slpA promoters in intracellular protein production was studied using three reporter genes, beta-glucuronidase (gusA), luciferase (luc) and aminopeptidase N (pepN) in three different lactic acid bacteria hosts: Lactococcus lactis, Lactobacillus plantarum and Lactobacillus gasseri. The S-layer promoters were recognized in each strain and especially L. lactis and Lb. plantarum exhibited high levels of transcripts. The production kinetics of reporter proteins was studied as a function of growth. The GusA, Luc and PepN activities varied considerably among the lactic acid bacterial strains studied. The highest levels of beta-glucuronidase and luciferase activity were obtained in L. lactis. The level of GusA obtained in L. lactis corresponded to over 15% of the total cellular proteins. The highest level of aminopeptidase N activity was achieved in Lb. plantarum where PepN corresponded up to 28% of the total cellular proteins at the late exponential phase of growth. This level of PepN activity is 30-fold higher than that in Lb. helveticus, which is the species from which the pepN gene originates.

L31 ANSWER 8 OF 16 USPATFULL
AN 1998:85822 USPATFULL
TI Gene and protein applicable to the preparation of vaccines for rickettsia prowazekii and rickettsia typhi and the detection of both
IN Carl, Mitchell, San Diego, CA, United States
Dobson, Michael E., Rockville, MD, United States
Ching, Wei-Mei, Bethesda, MD, United States
Dasch, Gregory A., Wheaton, MD, United States
PA The United States of America as represented by the Secretary of the Navy, Washington, DC, United States (U.S. government)
PI US 5783441 19980721
AI US 1993-169927 19931220 (8)
RLI Continuation-in-part of Ser. No. US 1991-742128, filed on 9 Aug 1991, now abandoned
DT Utility
EXNAM Primary Examiner: Housel, James C.; Assistant Examiner: Shaver, Jennifer
LREP Spevack, A. David; Garvert, William C.
CLMN Number of Claims: 2
ECL Exemplary Claim: 1
DRWN 5 Drawing Figure(s); 3 Drawing Page(s)
LN.CNT 928

CAS INDEXING IS AVAILABLE FOR THIS PATENT.
AB All or part of the DNA sequence of the gene which encodes the S-layer protein of R. prowazekii as illustrated in Sequence ID No. 1 as well as a truncated identical piece of this gene in R. typhi as well as the 5' and 3' noncoding regions can be used for vaccination against typhus and spotted fever rickettsial infection or to diagnose the diseases caused by these bacteria. The invention is also accomplished by the deduced amino acid sequence of the S-layer protein of R. prowazekii derived from the DNA sequence of the encoding gene. Further, the invention includes the peptide or protein products based on all or parts of this gene.

L31 ANSWER 9 OF 16 USPATFULL
AN 1998:19813 USPATFULL
TI Vacuolating toxin-deficient H. pylori
IN Cover, Timothy L., Nashville, TN, United States
Blaser, Martin J., Nashville, TN, United States
PA Vanderbilt University, Nashville, TN, United States (U.S. corporation)
PI US 5721349 19980224
AI US 1994-200232 19940223 (8)
RLI Continuation-in-part of Ser. No. US 1992-841644, filed on 26 Feb 1992, now abandoned
DT Utility
EXNAM Primary Examiner: Sidberry, Hazel F.
LREP Needle & Rosenberg, P.C.
CLMN Number of Claims: 5
ECL Exemplary Claim: 1
DRWN No Drawings
LN.CNT 1466
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
AB An isolated nucleic acid encoding the Helicobacter pylori vacuolating toxin, consisting of the nucleotides 101 through 3964 of the nucleotide sequence defined in the Sequence Listing as SEQ ID NO:1 is provided. An isolated nucleic acid from Helicobacter pylori comprising the nucleotide sequence defined in the Sequence Listing as SEQ ID NO:3 is provided. Isolated nucleic acids that selectively hybridize with the nucleic acids of the invention are provided. Also provided is a genetically altered mutant strain of H. pylori that does not express a functional vacuolating toxin. Purified proteins encoded by the nucleic acids of the invention are provided. A composition comprising an immunogenic amount of a protein or mutant strain of the invention in a pharmaceutically acceptable carrier is provided. A method of immunizing a subject against infection by H. pylori, comprising administering to the subject an immunogenic composition of the invention is provided.

L31 ANSWER 10 OF 16 BIOSIS COPYRIGHT 2000 BIOSIS
AN 1997:115621 BIOSIS
DN PREV199799414824
TI Linker mutagenesis of the Caulobacter crescentus S-layer protein: Toward a definition of an N-terminal anchoring region and a C-terminal secretion signal and the potential for **heterologous** protein secretion.
AU Bingle, Wade H.; Nomellini, John F.; Smit, John (1)
CS (1) Dep. Microbiol. Immunol., Univ. B.C. No. 300, 6174 University Blvd., Vancouver, BC V6T 1Z3 Canada
SO Journal of Bacteriology, (1997) Vol. 179, No. 3, pp. 601-611. ISSN: 0021-9193.
DT Article
LA English
AB Linker insertion mutagenesis was used to modify the paracrystalline surface layer (S-layer) protein (RsaA) of the gram-negative bacterium

Caulobacter crescentus. Eleven unique BamHI linker insertions in the cloned rsaA gene were identified; at the protein level, these linker insertions introduced 4 to 6 amino acids at positions ranging from the extreme N terminus to the extreme C terminus of the 1,026-amino-acid RsaA protein. All linker-peptide insertions in the RsaA N terminus caused the secreted protein to be shed into the growth medium, suggesting that the RsaA N terminus is involved in cell surface anchoring. One linker-peptide insertion in the RsaA C terminus (amino acid 784) had no effect on S-layer biogenesis, while another (amino acid 907) disrupted secretion of the protein, suggesting that RsaA possesses a secretion signal lying C terminal to amino acid 784, near or including amino acid 907. Unlike extreme N- or C-terminal linker-peptide insertions, those more centrally located in the RsaA primary sequence had no apparent effect on S-layer biogenesis. By using a newly introduced linker-encoded restriction site, a 3' fragment of the rsaA gene encoding the last 242 C-terminal amino acids of the S-layer protein was expressed in C. crescentus from **heterologous** Escherichia coli lacZ transcription and translation initiation information. This C-terminal portion of RsaA was secreted into the growth medium, confirming the presence of a C-terminal secretion signal. The use of the RsaA C terminus for the secretion of **heterologous** proteins in C. crescentus was explored by fusing 109 amino acids of an envelope glycoprotein from infectious hematopoietic necrosis virus, a pathogen of salmonid fish, to the last 242 amino acids of the RsaA C terminus. The resulting hybrid protein was successfully secreted into the growth medium and accounted for 10% of total protein in a stationary-phase culture. Based on these results and features of the RsaA primary sequence, we propose that the C. crescentus S-layer protein is secreted by a type I secretion system, relying on a stable C-terminal secretion signal in a manner analogous to E. coli alpha-hemolysin, the first example of an S-layer protein secreted by such a pathway.

L31 ANSWER 11 OF 16 BIOSIS COPYRIGHT 2000 BIOSIS
AN 1998:1037 BIOSIS
DN PREV199800001037
TI Cell surface display of a Pseudomonas aeruginosa strain K pilin peptide within the paracrystalline S-layer of Caulobacter crescentus.
AU Bingle, Wade H.; Nomellini, John F.; Smit, John (1)
CS (1) Dep. Microbiol. Immunol., Univ. B.C., 300-6174 University Blvd., Vancouver, BC V6T 2Z3 Canada
SO Molecular Microbiology, (Oct., 1997) Vol. 26, No. 2, pp. 277-288. ISSN: 0950-382X.
DT Article
LA English
AB The paracrystalline surface (S)-layer of Caulobacter crescentus is composed of a single secreted protein (RsaA) that interlocks in a hexagonal pattern to completely envelop the bacterium. Using a genetic approach, we inserted a 12 amino acid peptide from Pseudomonas aeruginosa strain K pilin at numerous semirandom positions in RsaA. We then used an immunological screen to identify those sites that presented the inserted pilin peptide on the C. crescentus cell surface as a part of the S-layer. Eleven such sites (widely separated in the primary sequence) were identified, demonstrating for the first time that S-layers can be readily exploited as carrier proteins to display 'epitope-size' **heterologous** peptides on bacterial cell surfaces. Whereas intact RsaA molecules carrying a pilin peptide could always be found on the surface of C. crescentus regardless of the particular insertion site, introduction of the pilin peptide at 9 of the 11 sites resulted in some proteolytic cleavage of RsaA. Two types of proteolytic phenomena were observed. The first was characterized by a single cleavage within the pilin peptide insert with both fragments of the S-layer protein remaining anchored to the outer membrane. The other proteolytic phenomenon was characterized by cleavage of the S-layer protein at a point distant from the site of the pilin peptide insertion. This cleavage always occurred at

the same location in RsaA regardless of the particular insertion site, yielding a surface-anchored 26 kDa proteolytic fragment bearing the RsaA N-terminus; the C-terminal cleavage product carrying the pilin peptide was released into the growth medium. When the results of this work were combined with the results of a previous study, the RsaA primary sequence could be divided into three regions with respect to the location of a peptide insertion and its effect on S-layer biogenesis: (i) insertions in the extreme N-terminus of RsaA either produce no apparent effect on S-layer biogenesis or disrupt surface-anchoring of the protein; (ii) insertions in the extreme C-terminus either produce no apparent effect on S-layer biogenesis or disrupt protein secretion; and (iii) insertions more centrally located in the protein either have no apparent effect on S-layer biogenesis or result in proteolytic cleavage of RsaA. These data are discussed in relation to our previous assignment of the RsaA N- and C-terminus as regions that are important for surface anchoring and secretion respectively.

L31 ANSWER 12 OF 16 USPATFULL
AN 96:23036 USPATFULL
TI Bacterial surface protein expression
IN Smit, John, Richmond, Canada
Bingle, Wade H., Vancouver, Canada
PA The University of British Columbia, Vancouver, Canada (non-U.S. corporation)
PI US 5500353 I9960319
AI US 1994-194290 19940209 (8)
RLI Continuation-in-part of Ser. No. US 1992-895367, filed on 9 Jun 1992, now abandoned
DT Utility
EXNAM Primary Examiner: Wax, Robert A.; Assistant Examiner: Kim, Hyosuk
LREP Shlesinger, Arkwright & Garvey
CLMN Number of Claims: 5
ECL Exemplary Claim: 1
DRWN 11 Drawing Figure(s); 9 Drawing Page(s)
LN.CNT 898
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
AB This invention provides a bacterium having an S-layer modified such that the bacterium S-layer protein gene contains one or more in-frame sequences coding for one or more **heterologous** polypeptides and, the S-layer is a fusion product of the S-layer protein and the **heterologous** polypeptide. The bacterium is preferably a Caulobacter which may be cultured as a film in a bioreactor or may be used to present an antigenic epitope to the environment of the bacterium. This invention also provides a method of expressing and presenting to the environment of a Caulobacter, a polypeptide that is **heterologous** to the S-layer of Caulobacter which comprises cloning a coding sequence for the polypeptide in-frame into an S-layer protein gene of Caulobacter whereby the polypeptide is expressed and presented on the surface of the Caulobacter as a fusion product of the S-layer protein and the polypeptide in the S-layer of the Caulobacter.

L31 ANSWER 13 OF 16 EMBASE COPYRIGHT 2000 ELSEVIER SCI. B.V.DUPLICATE 3
AN 96100429 EMBASE
DN 1996100429
TI Expression and purification of the crystalline **surface layer protein** of Rickettsia typhi.
AU Hahn M.-J.; Chang W.-H.
CS Department of Microbiology, College of Medicine, Kon-Kuk University,Danwol-Dong, Choongju 380-701, Korea, Republic of
SO Microbiology and Immunology, (1996) 40/3 (233-236).
ISSN: 0385-5600 CODEN: MIIMDV

CY Japan
DT Journal; Article
FS 004 Microbiology
LA English
SL English
AB The crystalline surface layer (S-layer) protein (SLP) of Rickettsia typhi is known as the protective antigen against murine typhus. We previously reported a cloning and sequence analysis of the SLP gene of R. typhi (slpT) and showed that the open reading frame of this gene encodes both the SLP and a 32-kDa protein. To express only the SLP from this gene, the putative signal sequence and the 32-kDa protein portion were removed from the slpT. This protein was expressed in Escherichia coli as a **fusion protein**, consisting of the SLP and maltose binding protein. The recombinant protein reacted strongly with polyclonal antiserum of a patient with murine typhus.

L31 ANSWER 14 OF 16 BIOTECHDS COPYRIGHT 2000 DERWENT INFORMATION LTD
AN 1995-11949 BIOTECHDS
TI Host cell expressing **surface layer protein**; Bordetella pertussis P69 antigen, pertussis toxin, tetanus toxin fragment-C, Escherichia coli heat-labile toxin B-subunit or E. coli K88 antigen surface display on Bacillus sphaericus
AU Deblaere R Y; Desomer J; Dhaese P
PA Solvay
PI WO 9519371 20 Jul 1995
AI WO 1995-EP147 13 Jan 1995
PRAI GB 1994-650 14 Jan 1994
DT Patent
LA English
OS WPI: 1995-263827 [34]
AB A new host cell has a surface layer (S-layer) containing a **fusion protein**, composed of at least sufficient S-layer protein for assembly, and a **heterologous** protein fragment fused to the C-terminus or N-terminus, which is then presented on the outer surface of the cell. The following are also new: DNA containing a promoter (e.g. a Bacillus sp. S-layer protein promoter, such as the P1 promoter of Bacillus sphaericus P-1 (LMG P-13855)) operably linked to a sequence encoding a signal peptide and the **fusion protein**; a promoter with specified -35 and -10 regions; an expression vector with the promoter and a downstream cloning site; and a process for transformation of B. sphaericus P-1 by harvesting cells at late stationary phase, mixing with DNA, and carrying out electroporation. The **heterologous** protein may be a virus, bacterium, fungus, yeast or parasite antigen, e.g. Bordetella pertussis P69 antigen, pertussis toxin or a subunit, tetanus toxin fragment-C, Escherichia coli heat-labile toxin B-subunit or E. coli K88 antigen. Cells presenting the **fusion protein** on their surface may be used as a recombinant vaccine. (95pp)

L31 ANSWER 15 OF 16 BIOSIS COPYRIGHT 2000 BIOSIS
AN 1995:292292 BIOSIS
DN PREV199598306592
TI Insertion of **heterologous** peptides within the **surface-layer protein** of Caulobacter crescentus.
AU Nomellini, J. F.; Le, K. D.; Bingle, W. H.; Smit, J.
CS Dep. Microbiol. Immunol., Univ. British Columbia, Vancouver, B.C. Canada
SO Abstracts of the General Meeting of the American Society for Microbiology, (1995) Vol. 95, No. 0, pp. 525.
Meeting Info.: 95th General Meeting of the American Society for Microbiology Washington, D.C., USA May 21-25, 1995
ISSN: 1060-2011.
DT Conference

LA English

L31 ANSWER 16 OF 16 BIOTECHDS COPYRIGHT 2000 DERWENT INFORMATION LTD
AN 1994-13835 BIOTECHDS
TI Alkaline phosphatase and a cellulase reporter protein are not exported from the cytoplasm when fused to large N-terminus portions of the Caulobacter crescentus surface (S)-layer protein; reporter protein secretion
AU Bingle W H; *Smit J
CS Univ.British-Columbia
LO Department of Microbiology and Immunology, University of British Columbia, Vancouver, BC, Canada V6T 1Z3.
SO Can.J.Microbiol.; (1994) 40, 9, 777-82
COOEN: CJMIAZ
DT Journal
LA English
AB Hybrid proteins were created by linking alkaline phosphatase (PhoA, EC-3.1.3.1) or a cellulase (EC-3.2.1.4) reporter (delta-CenA) to 4 large N-terminal portions of the Caulobacter crescentus surface (S)-layer protein (RsaA). 3 Of the sites (amino acids 189, 220, 315) were selected on the basis of experiments that suggested that the first 250-250 amino acids of RsaA could mediate export of PhoA from the cytoplasm, while the 4th lay 21 amino acids from the C-terminus. Expression of all fusions except rasA(315):delta-cenA and rsaA(315):phoA was toxic to C. crescentus JS4001. Truncated RsaA peptides lacking their reporter domains were nontoxic. RsaA(delta-C21) was not secreted nor prone to intracellular assimilation. None of the gene fusions was toxic when expressed by Escherichia coli DH5-alpha. Although C. crescentus tolerated the expression of rasA(315):delta-cenA and rsaA(315):phoA, the encoded hybrid proteins were not exported in significant quantities from the cytoplasm. The results suggest that the entire native S-layer protein may be required to properly interact with the RsaA secretion machinery. (23 ref)

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(FILE 'HOME' ENTERED AT 16:15:00 ON 11 DEC 2000)

FILE 'EMBASE, MEDLINE, BIOSIS, BIOTECHDS, USPATFULL, CAPLUS, AGRICOLA, LIFESCI, CONFSCI' ENTEREO AT 16:15:49 ON 11 DEC 2000

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                E LUBITZ WERNER/AU
L1          692 S E1-E3
L2            0 S L1 AND FUSION PROTEIN (5A) BACILLUS
L3           26 S L1 AND (FUSION PROTEIN OR HETEROLOGOUS PROTEIN)
L4           12 DUP REM L3 (14 OUPLICATES REMOVEO)
L5           36 S L1 ANO S LAYER PROTEIN
L6           14 DUP REM L5 (22 OUPLICATES REMOVED)
                E SLEYTR UWE/AU
L7          735 S E1 OR E3 OR E4 OR E5
L8           15 S L7 AND (FUSION PROTEIN OR HETEROLOGOUS)
L9            4 DUP REM L8 (11 DUPLICATES REMOVED)
L10         213 S L7 AND S LAYER PROTEIN
L11           6 S L10 AND (FUSION PROTEIN OR HETEROLOGOUS)
                E KUEN BEATRIX/AU
L12          60 S E1-E3
L13           5 S L12 AND (FUSION PROTEIN OR HETEROLOGOUS)
L14          41 S L12 AND S LAYER PROTEIN
L15          14 DUP REM L14 (27 DUPLICATES REMOVEO)
                E TRUPPE MICHAELA/AU
L16           9 S E1-E3
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L17            8 DUP REM L16 (1 DUPLICATE REMOVED)
                 E HOWORKA STEFAN/AU
L18           24 S E1 OR E3 OR E4
L19           13 S L18 AND S LAYER PROTEIN
L20            4 DUP REM L19 (9 DUPLICATES REMOVED)
                 E RESCH STEPHANKA/AU
L21            6 S E1 OR E2 OR E4
L22            4 DUP REM L21 (2 DUPLICATES REMOVED)
                 E SCHROLL GERHARD/AU
L23            8 S E3
L24            5 DUP REM L23 (3 DUPLICATES REMOVED)
                 E SARA MARGIT/AU
L25          119 S E3
L26           70 S L25 AND S LAYER PROTEIN
L27            0 S L26 AND FUSION PROTEIN
L28          916 S S LAYER PROTEIN
L29          345 S SURFACE LAYER PROTEIN
L30           24 S L29 AND (FUSION PROTEIN OR HETEROLOGOUS)
L31           16 DUP REM L30 (8 DUPLICATES REMOVED)

=> s l28 and (fusion protein or heterologous)

L32          97 L28 AND (FUSION PROTEIN OR HETEROLOGOUS)

=> s l32 and (immunogenic or epitope or enzyme? or cytokine or antigenic or
metal binding  or antibody binding)

   6 FILES SEARCHED...
L33          37 L32 AND (IMMUNOGENIC OR EPITOPE OR ENZYME? OR CYTOKINE OR ANTIG
                ENIC OR METAL BINDING OR ANTIBODY BINDING)

=> dup rem l33

PROCESSING COMPLETED FOR L33
L34           18 DUP REM L33 (19 DUPLICATES REMOVED)

=> d bib ab 1-18


L34   ANSWER 1 OF 18  BIOTECHDS COPYRIGHT 2000 DERWENT INFORMATION LTD
AN    2000-05880  BIOTECHDS
TI    Cleavage of Caulobacter produced recombinant fusion proteins useful for
      producing vaccine peptides;
         including recombinant human and animal therapeutic antibiotic and
         vaccine peptides, enzymes, protein polymers and
         antibacterial enzymes for foodstuffs
AU    Smit J
PA    Univ.British-Columbia
LO    Vancouver, British Columbia, Canada.
PI    WO 2000004170 27 Jan 2000
AI    WO 1999-CA637 14 Jul 1999
PRAI  CA 1998-2237704 14 Jul 1998
DT    Patent
LA    English
OS    WPI: 2000-182434 [16]
AB    Cleaving a fusion consisting of a Caulobacter S-layer
    protein (containing the C-terminal secretion signal) and a second
      component heterologous to Caulobacter, using an acid solution,
      is claimed.  Also claimed are: preparing a DNA construct for expression
      of the fusion protein; and producing a fusion
    protein using the DNA construct.  The method is useful for
      producing pure proteins including recombinant human and animal
      therapeutic antibiotic and vaccine peptides, enzymes, protein
      polymers and antibacterial enzymes for foodstuffs.  The method
      enables economic production of pure proteins, and it reduces the number
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of purification steps required following fermentation. The aspartate-proline dipeptide is located between the first and second components or adjacent junction between components. The acid solution has a pH range of 1.5-2.5 or 1.65-2.35. The method is carried out at a temperature of 30-50 deg. Cleaved products are preferably separated from the **fusion protein**. Oligonucleotides involved in the isolation of polynucleotides are prepared using conventional solid phase techniques. (33pp)

L34 ANSWER 2 OF 18 BIOTECHDS COPYRIGHT 2000 DERWENT INFORMATION LTD
AN 1999-04719 BIOTECHDS
TI Producing S-layer proteins in Gram-negative bacteria or eukaryotes; for use as recombinant vaccine
AU Lubitz W
PA Lubitz W
LO Vienna, Austria.
PI DE 19732829 4 Feb 1999
AI DE 1997-1032829 30 Jul 1997
PRAI DE 1997-1032829 30 Jul 1997
DT Patent
LA German
OS WPI: 1999-122189 [11]
AB A means of producing **S-layer protein** (I) is claimed. It involves transforming a Gram-negative prokaryotic cell with a nucleic acid that encodes (I) linked to a signal peptide that encodes a protein which causes integration of (I) into the external or cytoplasmic membrane, or secretion of (I) into the periplasmic space or extracellular medium. The bacterium is then cultured, and (I) recovered from the membrane, periplasmic space, or medium. Alternatively a eukaryotic cell can be used as the host, in which case the signal peptide promotes integration of (I) into the cytoplasmic membrane, or an organelle, or induces secretion of (I) into the extracellular medium. Also claimed is a nucleic acid (II) that encodes (I) and the signal peptide, optionally including **heterologous** peptide inserts. The claims also cover a vector containing (II), and Gram-positive prokaryotic or eukaryotic cells transformed by that vector (e.g. plasmid pMAL-A used to transform Escherichia coli DH5-alpha. (I) are useful as vaccines, reactors, and universal carrier molecules. (33pp)

L34 ANSWER 3 OF 18 USPATFULL DUPLICATE 2
AN 1999:137013 USPATFULL
TI Expression and secretion of **heterologous** polypeptides from caulobacter
IN Smit, John, Richmond, Canada
Bingle, Wade H., Vancouver, Canada
Nomellini, John F., Richmond, Canada
PA The University of British Columbia, Canada (non-U.S. corporation)
PI US 5976864 19991102
AI US 1996-614377 19960312 (8)
RLI Continuation-in-part of Ser. No. US 1994-194290, filed on 9 Feb 1994, now patented, Pat. No. US 5500353 which is a continuation-in-part of Ser. No. US 1992-895367, filed on 9 Jun 1992, now abandoned
DT Utility
EXNAM Primary Examiner: Wax, Robert A.; Assistant Examiner: Nashed, Nashaat T.
LREP Fish & Richardson P.C.
CLMN Number of Claims: 14
ECL Exemplary Claim: 2
DRWN 14 Drawing Figure(s); 13 Drawing Page(s)
LN.CNT 1609
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
AB DNA constructs are provided which code for at least the extreme

C-terminal amino acids of the rsaA protein of Caulobacter crescentus fused with **heterologous** polypeptides. Baterial cells containing, or which express the DNA constructs and secrete the resulting protein are also provided. Chimeric proteins including the C-terminal amino acids of the rsaA protein are provided, including chimeric proteins comprising **antigenic** epitopes of the Infectious Hematopoietic Necrosis Virus.

L34 ANSWER 4 OF 18 USPATFULL
AN 1999:24489 USPATFULL
TI Expression of surface layer proteins
IN Deblaere, Rolf Y., Waarschoot, Belgium
Desomer, Jan, Drongen, Belgium
Dhaese, Patrick, Drongen, Belgium
PA Solvay (Societe Anonyme), Brussels, Belgium (non-U.S. corporation)
PI US 5874267 19990223
WO 9519371 19950720
AI US 1996-682517 19960917 (8)
WO 1995-EP147 19950113
19960917 PCT 371 date
19960917 PCT 102(e) date
PRAI GB 1994-650 19940114
OT Utility
EXNAM Primary Examiner: Hutzell, Paula K.; Assistant Examiner: Bakalyar, Heather A.
LREP McOermott, Will & Emery
CLMN Number of Claims: 1
ECL Exemplary Claim: 1
DRWN 41 Drawing Figure(s); 37 Drawing Page(s)
LN.CNT 2742
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
AB A host cell which is provided with a S-layer comprising a fusion polypeptide consisting essentially of:

(a) at least sufficient of a **S-layer protein** for a S-layer composed thereof to assemble, and

(b) a **heterologous** polypeptide which is fused to either the carboxy terminus of (a) or the amino terminus of (a) and which is thereby presented on the outer surface of the said cell; can be used as a vaccine, for screening for proteins and antigens and as a support for immobilizing an **enzyme**, peptide or antigen. A process of transforming B. Sphaericus cells comprising electroporation is also provided.

L34 ANSWER 5 OF 18 EMBASE COPYRIGHT 2000 ELSEVIER SCI. B.V.DUPLICATE 3
AN 1999051248 EMBASE
TI The expression signals of the Lactobacillus brevis slpA gene direct efficient **heterologous** protein production in lactic acid bacteria.
AU Kahala M.; Palva A.
CS A. Palva, Dept. of Basic Veterinary Sciences, Faculty of Veterinary Medicine, University of Helsinki, P.O. Box 57, 00014 Helsinki, Finland. airi.palva@helsinki.fi
SO Applied Microbiology and Biotechnology, (1999) 51/1 (71-78).
Refs: 42
ISSN: 0175-7598 CODEN: AMBIDG
CY Germany
OT Journal; Article
FS 004 Microbiology
LA English
SL English
AB A cassette based on the expression signals of the Lactobacillus brevis surface (s)-**layer protein** gene (slpA) was constructed. The low-copy-number vector pKTH2095, derived from pGK12, was

used as the cloning vector. The efficiency of slpA promoters in intracellular protein production was studied using three reporter genes, .beta.-glucuronidase (gusA), luciferase (luc) and aminopeptidase N (pepN) in three different lactic acid bacteria hosts: Lactococcus lactis, Lactobacillus plantarum and Lactobacillus gasseri. The S- layer promoters were recognized in each strain and especially L. lactis and Lb. plantarum exhibited high levels of transcripts. The production kinetics of reporter proteins was studied as a function of growth. The GusA, Luc and PepN activities varied considerably among the lactic acid bacterial strains studied. The highest levels of .beta.-glucuronidase and luciferase activity were obtained in L. lactis. The level of GusA obtained in L. lactis corresponded to over 15% of the total cellular proteins. The highest level of aminopeptidase N activity was achieved in Lb. plantarum where PepN corresponded up to 28% of the total cellular proteins at the late exponential phase of growth. This level of PepN activity is 30-fold higher than that in Lb. helveticus, which is the species from which the pepN gene originates.

L34 ANSWER 6 OF 18 LIFESCI COPYRIGHT 2000 CSA
AN 2000:17017 LIFESCI
TI Expression of surface layer proteins
AU Deblaere, R.; Desomer, J.; Dhaese, P.
CS Solvay (Societe Anonyme)
SO (19990223) . US Patent: 5874267; US CLASS: 435/173.6; 435/172.3..
DT Patent
FS W3
LA English
SL English
AB A host cell which is provided with a S-layer comprising a fusion polypeptide consisting essentially of: (a) at least sufficient of a **S-layer protein** for a S-layer composed thereof to assemble, and (b) a **heterologous** polypeptide which is fused to either the carboxy terminus of (a) or the amino terminus of (a) and which is thereby presented on the outer surface of the said cell; can be used as a vaccine, for screening for proteins and antigens and as a support for immobilizing an **enzyme**, peptide or antigen. A process of transforming B. sphaericus cells comprising electroporation is also provided.

L34 ANSWER 7 OF 18 USPATFULL
AN 1998:85822 USPATFULL
TI Gene and protein applicable to the preparation of vaccines for rickettsia prowazekii and rickettsia typhi and the detection of both
IN Carl, Mitchell, San Diego, CA, United States
Dobson, Michael E., Rockville, MD, United States
Ching, Wei-Mei, Bethesda, MD, United States
Dasch, Gregory A., Wheaton, MD, United States
PA The United States of America as represented by the Secretary of the Navy, Washington, DC, United States (U.S. government)
PI US 5783441 19980721
AI US 1993-169927 19931220 (8)
RLI Continuation-in-part of Ser. No. US 1991-742128, filed on 9 Aug 1991, now abandoned
DT Utility
EXNAM Primary Examiner: Housel, James C.; Assistant Examiner: Shaver, Jennifer
LREP Spevack, A. David; Garvert, William C.
CLMN Number of Claims: 2
ECL Exemplary Claim: 1
DRWN 5 Drawing Figure(s); 3 Drawing Page(s)
LN.CNT 928
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
AB All or part of the DNA sequence of the gene which encodes the **S -layer protein** of R. prowazekii as illustrated in Sequence ID No. 1 as well as a truncated identical piece of this gene in

R. typhi as well as the 5' and 3' noncoding regions can be used for vaccination against typhus and spotted fever rickettsial infection or to diagnose the diseases caused by these bacteria. The invention is also accomplished by the deduced amino acid sequence of the **S-layer protein** of R. prowazekii derived from the DNA sequence of the encoding gene. Further, the invention includes the peptide or protein products based on all or parts of this gene.

L34 ANSWER 8 OF 18 BIOTECHDS COPYRIGHT 2000 DERWENT INFORMATION LTD
AN 1997-12704 BIOTECHDS
TI New DNA containing sequence for C-terminal region of Caulobacter **S-layer protein**; e.g. Caulobacter crescentus RsaA protein and antigen **fusion protein** expression and surface display, for use as a recombinant vaccine
AU Smit J; Bingle W H; Nomellini J F
PA Univ.British-Columbia
LO Vancouver, British Columbia, Canada.
PI WO 9734000 18 Sep 1997
AI WO 1997-CA167 10 Mar 1997
PRAI US 1996-614377 12 Mar 1996
DT Patent
LA English
OS WPI: 1997-470880 [43]
AB A new DNA construct contains at least 1 restriction site for facilitating insertion of DNA upstream of a sequence encoding a C-terminal region (at least 82 amino acids) of a Caulobacter sp. **S-layer protein** (e.g. Caulobacter crescentus RsaA protein amino acids 945-1026, 850-1026 or 782-1026). The construct may also contain a **heterologous** gene (encoding a protein of up to 60 or 200 amino acids) upstream from the S-layer sequence, and an operably linked promoter. The recombinant host cell may form a surface layer containing the **heterologous** protein. Caulobacter spp. containing the new DNA construct are particularly useful as live recombinant vaccines (where the **heterologous** protein is an antigen). They may also be used for production of e.g. ligands, **enzymes** or other proteins. All known Caulobacter spp. are harmless, and stable in outdoor environments, including water (for use as fish vaccines) or soil. They are well suited for growing in biofilm fermentors, and produce an S-layer, which is an ideal antigen presentation system, at a high level. (58pp)

L34 ANSWER 9 OF 18 EMBASE COPYRIGHT 2000 ELSEVIER SCI. B.V.DUPLICATE 4
AN 97348869 EMBASE
DN 1997348869
TI Cell-surface display of a Pseudomonas aeruginosa strain K pilin peptide within the paracrystalline S-layer of Caulobacter crescentus.
AU Bingle W.H.; Nomellini J.F.; Smit J.
CS J. Smit, Dept. Microbiology and Immunology, University of British Columbia, 300-6174 University Boulevard, Vancouver, BC V6T 1Z3, Canada. jsmit@unixg.ubc.ca
SO Molecular Microbiology, (1997) 26/2 (277-288).
Refs: 25
ISSN: 0950-382X CODEN: MOMIEE
CY United Kingdom
DT Journal; Article
FS 004 Microbiology
LA English
SL English
AB The paracrystalline surface (S)-layer of Caulobacter crescentus Is composed of a single secreted protein (RsaA) that interlocks in a hexagonal pattern to completely envelop the bacterium. Using a genetic

approach, we inserted a 12 amino acid peptide from Pseudomonas aeruginosa strain K pilin at numerous semirandom positions in RsaA. We then used an Immunological screen to identify those sites that presented the inserted pilin peptide on the C. crescentus cell surface as a part of the S-layer. Eleven such sites (widely separated in the primary seguence) were identified, demonstrating for the first time that S-layers can be readily exploited as carrier proteins to display **'epitope-size' heterologous** peptides on bacterial cell surfaces. Whereas intact RsaA molecules carrying a pilin peptide could always be found on the surface of C. crescentus regardless of the particular insertion site, introduction of the pilin peptide at 9 of the 11 sites resulted In some proteolytic cleavage of RsaA. Two types of proteolytic phenomena were observed. The first was characterized by a single cleavage within the pilin peptide insert with both fragments of the **S-layer protein** remaining anchored to the outer membrane. The other proteolytic phenomenon was characterized by cleavage of the **S-layer protein** at a point distant from the site of the pilin peptide insertion. This cleavage always occurred at the same location In RsaA regardless of the particular insertion site, yielding a surface-anchored 26 kDa proteolytic fragment bearing the RsaA N-terminus; the C-terminal cleavage product carrying the pilin peptide was released into the growth medium. When the results of this work were combined with the results of a previous study, the RsaA primary sequence could be divided into three regions with respect to the location of a peptide insertion and its effect on S-layer biogenesis: (i) insertions in the extreme N-terminus of RsaA either produce no apparent effect on S-layer biogenesis or disrupt surface-anchoring of the protein; (ii) insertions in the extreme C-terminus either produce no apparent effect on S-layer biogenesis or disrupt protein secretion; and (iii) insertions more centrally located in the protein either have no apparent effect on S-layer biogenesis or result in proteolytic cleavage of RsaA. These data are discussed in relation to our previous assignment of the RsaA N- and C-terminus as regions that are important for surface anchoring and secretion respectively.

L34 ANSWER 10 OF 18 BIOTECHDS COPYRIGHT 2000 DERWENT INFORMATION LTD
AN 1997-05711 BIOTECHDS
TI High level **heterologous** protein production in Lactococcus and Lactobacillus using a new secretion system based on the Lactobacillus brevis S-layer signals;
plasmid pKTH2121 for beta-lactamase production in Lactococcus lactis, Lactobacillus brevis, Lactobacillus plantarum, Lactobacillus gasseri and Lactobacillus casei
AU Savijoki K; Kahala M; *Palva A
CS Food-Res.Inst.Jokioinen
LO Agricultural Research Center of Finland, Food Research Institute, Jokioinen 31600, Finland.
Email: airi.palva@mtt.fi
SO Gene; (1997) 186, 2, 255-62
CODEN: GENED6 ISSN: 0378-1119
DT Journal
LA English
AB A secretion DNA cassette (plasmid pKTH2121) based on the expression and secretion signals of a **S-layer protein** (SlpA) from Lactobacillus brevis was constructed for high level **heterologous** Escherichia coli beta-lactamase (EC-3.5.2.6) protein production in Lactococcus lactis (MG1614), Lactobacillus brevis, Lactobacillus plantarum, Lactobacillus gasseri and Lactobacillus casei using a low-copy-number plasmid derived from plasmid pGK12. To determine whether pH control improved the stability and production of beta-lactamase, L. lactis and L. brevis were grown at 30 and 37 deg, respectively, with 100 rpm in a fermenter with constant pH (pH 5.5). The

highest **enzyme** yield was obtained in L. lactis (80 mg/l) and L. brevis. Results indicated a wide applicability of the L. brevis SlpA signals for efficient protein production and secretion in lactic acid bacteria. (36 ref)

L34 ANSWER 11 OF 18 USPATFULL DUPLICATE 5
AN 96:23036 USPATFULL
TI Bacterial surface protein expression
IN Smit, John, Richmond, Canada
Bingle, Wade H., Vancouver, Canada
PA The University of British Columbia, Vancouver, Canada (non-U.S. corporation)
PI US 5500353 19960319
AI US 1994-194290 19940209 (8)
RLI Continuation-in-part of Ser. No. US 1992-895367, filed on 9 Jun 1992, now abandoned
DT Utility
EXNAM Primary Examiner: Wax, Robert A.; Assistant Examiner: Kim, Hyosuk
LREP Shlesinger, Arkwright & Garvey
CLMN Number of Claims: 5
ECL Exemplary Claim: 1
DRWN 11 Drawing Figure(s); 9 Drawing Page(s)
LN.CNT 898
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
AB This invention provides a bacterium having an S-layer modified such that
the bacterium **S-layer protein** gene
contains one or more in-frame sequences coding for one or more **heterologous** polypeptides and, the S-layer is a fusion product of the **S-layer protein** and the **heterologous** polypeptide. The bacterium is preferably a Caulobacter which may be cultured as a film in a bioreactor or may be used to present an **antigenic epitope** to the environment of the bacterium. This invention also provides a method of expressing and presenting to the environment of a Caulobacter, a polypeptide that is **heterologous** to the S-layer of Caulobacter which comprises cloning a coding sequence for the polypeptide in-frame into an **S-layer protein** gene of Caulobacter whereby the polypeptide is expressed and presented on the surface of the Caulobacter as a fusion product of the **S-layer protein** and the polypeptide in the S-layer of the Caulobacter.

L34 ANSWER 12 OF 18 LIFESCI COPYRIGHT 2000 CSA
AN 97:61228 LIFESCI
TI Bacterial surface protein expression
CS UNIVERSITY OF BRITISH COLUMBIA
SO (1996) . US Patent 5500353; US Cl. 435/69.1 424/192.1 424/197.11 435/69.3 435/69.7 435/177 435/209 435/252.3 514/6 530/350 530/395 530/400 536/22.1 536/23.1 536/23.4 536/23.7.
DT Patent
FS W2; A
LA English
AB This invention provides a bacterium having an S-layer modified such that the bacterium **S-layer protein** gene contains one or more in-frame sequences coding for one or more **heterologous** polypeptides and, the S-layer is a fusion product of the **S-layer protein** and the **heterologous** polypeptide. The bacterium is preferably a Caulobacter which may be cultured as a film in a bioreactor or may be used to present an **antigenic epitope** to the environment of the bacterium. This invention also provides a method of expressing and presenting to the environment of a Caulobacter, a polypeptide that is **heterologous** to the S-layer of Caulobacter which comprises cloning a coding sequence for the polypeptide in-frame into an **S-layer**

protein gene of Caulobacter whereby the polypeptide is expressed and presented on the surface of the Caulobacter as a fusion product of the **S-layer protein** and the polypeptide in the S-layer of the Caulobacter.

L34 ANSWER 13 OF 18 CAPLUS COPYRIGHT 2000 ACS
AN 1995:863580 CAPLUS
DN 123:278076
TI Sequence of the surface layer protein of Bacillus sphaericus P-1 and construction of functional fusion polypeptides
IN Deblaere, Rolf Y.; Desomer, Jan; Dhaese, Patrick
PA Solvay et Cie., Belg.
SO PCT Int. Appl., 95 pp.
CODEN: PIXXD2
DT Patent
LA English
FAN.CNT 1

	PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
PI	WO 9519371	A2	19950720	WO 1995-EP147	19950113
	WO 9519371	A3	19951116		
	W: JP, US				
	RW: AT, BE, CH, DE, DK, ES, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE				
	EP 738278	A1	19961023	EP 1995-908207	19950113
	R: AT, BE, CH, DE, DK, ES, FR, GB, IT, LI, NL, SE				
	JP 09508012	T2	19970819	JP 1995-518850	19950113
	US 5874267	A	19990223	US 1996-682517	19960917
PRAI	GB 1994-650	19940114			
	WO 1995-EP147	19950113			

AB A host cell which is provided with an S-layer comprising a fusion polypeptide consisting essentially of: (a) at least a sufficient portion of an **S-layer protein** for an S-layer to assemble, and (b) a **heterologous** polypeptide which is fused to either the C-terminus of (a) or the N-terminus of (a) and which is thereby presented on the outer surface of the cell. The fusion polypeptide can be used as a vaccine, for screening for proteins and antigens, and as a support for immobilizing an **enzyme**, peptide or antigen. Thus, the slp gene of Bacillus sphaericus strain P-1 was isolated and cloned to yield a 4.6-kb region contg. a 3756-bp open reading frame encoding 1252 amino acid residues. The SLP protein is glycosylated and contains an N-terminal signal peptide region. The slp promoter contains 3 different transcription initiation sites at positions -184, -340, and -385 with respect to the start codon. The latter 2 sites are repressed by calcium, whereas the first P1 site is independent of any neg. effect. Cloning of internal fragments of the slp gene fused to promoterless nptII gene in B. sphaericus P-1 by electroporation indicated that the C-terminal portion of SLP are dispensable for viability of P-1 cells, whereas the N-terminal part (esp. residues 31-269) are abs. required. Fusion of reporter proteins NPTII and the sol. fragment of the subunit S1 of toxin produced by Bordetella pertussis to the C-terminus of truncated SLPs yielded fusion polypeptides that assembly into a functional S-layer and in which the reporter proteins retain their enzymic activity.

L34 ANSWER 14 OF 18 EMBASE COPYRIGHT 2000 ELSEVIER SCI. B.V.DUPLICATE 6
AN 95190761 EMBASE
DN 1995190761
TI Segmental conservation of sapA sequences in type B Campylobacter fetus cells.
AU Dworkin J.; Tummuru M.K.R.; Blaser M.J.
CS Division of Infectious Diseases, A-3310 Medical Center North, Vanderbilt

Univ. School of Medicine,Nashville, TN 37232-2605, United States
SO Journal of Biological Chemistry, (1995) 270/25 (15093-15101).
ISSN: 0021-9258 CODEN: JBCHA3
CY United States
DT Journal; Article
FS 004 Microbiology
029 Clinical Biochemistry
LA English
SL English
AB Campylobacter fetus cells may exist as either of two defined serogroups (type A or B) based on their lipopolysaccharide (LPS) composition. Wild-type swains contain surface array proteins (S-layer proteins) that have partial **antigenic** cross-reactivity but bind exclusively to LPS from homologous (type A or B) cells. Type A cells possess 8 homologs of sapA, which encodes a 97- kDa **S-layer protein**; the gene products of these homologs have a conserved N terminus of 184 amino acids. To further explore the structural relationships between the C. fetus S-layer proteins and their encoding genes, we sought to clone and express an **S-layer protein** from type B strain 84-91. The cloned type B gene (sapB) was similar in structure to the previously cloned type A gene (sapA) and encoded a full-length 936-amino acid (97-kDa) **S-layer protein**. Sequence analysis of sapB indicated that the conserved N-terminal encoding region in sapA was absent but that the remainder of the ORF (encoding 751 amino acids) was identical to that of sapA in spite of the nonconserved nature of this region among sapA homologs. Noncoding sequences hath 300 base pairs 5' and 1000 base pairs 3' to the sapB and sapA ORFs, including the sapA promoter and transcriptional terminator sequences, were essentially identical. Southern analyses revealed that the sapB N-terminal encoding region was conserved in multiple copies in type B strains but was absent in type A strains. Recombinant sapA and sapB products bound to a substantially greater degree to cells of the homologous LPS type compared with the **heterologous** LPS type, indicating that the conserved sapA- and sapB- encoded N termini are critical for LPS binding specificity. The parallel genetic organization and identity at the nucleotide level in both coding and noncoding regions for sap homologs in types A and B cells indicates the necessity of both homolog conservation and high fidelity DNA replication in the biology of sap diversity.

L34 ANSWER 15 OF 18 BIOTECHDS COPYRIGHT 2000 DERWENT INFORMATION LTD
AN 1994-13835 BIOTECHDS
TI Alkaline phosphatase and a cellulase reporter protein are not exported from the cytoplasm when fused to large N-terminus portions of the Caulobacter crescentus surface (S)-**layer protein**; reporter protein secretion
AU Bingle W H; *Smit J
CS Univ.British-Columbia
LO Department of Microbiology and Immunology, University of British Columbia, Vancouver, BC, Canada V6T 1Z3.
SO Can.J.Microbiol.; (1994) 40, 9, 777-82
CODEN: CJMIAZ
DT Journal
LA English
AB Hybrid proteins were created by linking alkaline phosphatase (PhoA, EC-3.1.3.1) or a cellulase (EC-3.2.1.4) reporter (delta-CenA) to 4 large N-terminal portions of the Caulobacter crescentus surface (S)-**layer protein** (RsaA). 3 Of the sites (amino acids 189, 220, 315) were selected on the basis of experiments that suggested that the first 250-250 amino acids of RsaA could mediate export of PhoA from the cytoplasm, while the 4th lay 21 amino acids from the C-terminus. Expression of all fusions except rasA(315):delta-cenA and rsaA(315):phoA

was toxic to C. crescentus JS4001. Truncated RsaA peptides lacking their reporter domains were nontoxic. RsaA(delta-C21) was not secreted nor prone to intracellular assimilation. None of the gene fusions was toxic when expressed by Escherichia coli DH5-alpha. Although C. crescentus tolerated the expression of rasA(315):delta-cenA and rsaA(315):phoA, the encoded hybrid proteins were not exported in significant quantities from the cytoplasm. The results suggest that the entire native **S-layer protein** may be required to properly interact with the RsaA secretion machinery. (23 ref)

L34 ANSWER 16 OF 18 BIOTECHDS COPYRIGHT 2000 DERWENT INFORMATION LTD
AN 1994-04901 BIOTECHDS
TI Recombinant cellulase, endo-1,4-beta-D-xylanase and metallothionein and S-layer **fusion protein** production by vector expression in Caulobacter crescentus;
for metal recovery in waste-water, wood pulp treatment and as a fish recombinant vaccine
PA Univ.British-Columbia
PI CA 2090549 10 Dec 1993
AI CA 1993-2090549 26 Feb 1993
PRAI US 1992-895367 9 Jun 1992
DT Patent
LA English
OS WPI: 1994-066249 [09]
AB The following are claimed: (a) Caulobacter crescentus which has an **S-layer protein** gene containing 1 or more sequences encoding 1 or more functional **heterologous** proteins and where the S-layer is a fusion product of the **S-layer protein** and the **heterologous** protein; (b) a method of expressing and presenting a functional protein by cloning a DNA sequence encoding the protein in-frame into the **S-layer protein** gene of (a) and culturing the bacterium as a film in a fermentor; (c) a fusion product comprising an **S-layer protein** and 1 or more functional proteins sequences expressed by (b); (d) C. crescentus rsaA gene expressing **S-layer protein**, where the expressed part of the gene contains 1 or more **heterologous** restriction endonuclease sites; (e) a plasmid containing (d); and (f) a bacterium containing (e). The **heterologous** protein may be cellulase (EC-3.2.1.4) or endo-1,4-beta-D-xylanase (EC-3.2.1.8) capable of degrading wood, or a metallothionein. The **S-layer protein** bacterium system may be used to bind toxic metals in sewage, waste-water, etc., or for the treatment of wood pulp. It may also be used to produce **heterologous** protein for use in fish vaccines. (27pp)

L34 ANSWER 17 OF 18 EMBASE COPYRIGHT 2000 ELSEVIER SCI. B.V.DUPLICATE 8
AN 92092056 EMBASE
DN 1992092056
TI Reattachment of surface array proteins to Campylobacter fetus cells.
AU Yang L.; Pei Z.; Fujimoto S.; Blaser M.J.
CS Infectious Diseases Division, Department of Medicine, Vanderbilt Univ. Sch. of Med.,Nashville, TN 37232, United States
SO Journal of Bacteriology, (1992) 174/4 (1258-1267).
ISSN: 0021-9193 CODEN: JOBAAY
CY United States
DT Journal; Article
FS 004 Microbiology
LA English
SL English
AB Campylobacter fetus strains may be of serotype A or B, a property associated with lipopolysaccharide (LPS) structure. Wild-type C. fetus strains contain surface array proteins (S-layer proteins) that may be

extracted in water and that are critical for virulence. To explore the relationship of S-layer proteins to other surface components, we reattached S-layer proteins onto S- template cells generated by spontaneous mutation or by serial extractions of S+ cells with water. Reattachment occurred in the presence of divalent (Ba2+, Ca2+, Co2+, and Mg2+) but not monovalent (H+, NH4+, Na+, K+) or trivalent (Fe3+) cations. The 98-, 125-, 127-, and 149-kDa S-layer proteins isolated from strains containing type A LPS (type A **S-layer protein**) all reattached to S- template cells containing type A LPS (type A cells) but not to type B cells. The 98-kDa type B **S-layer protein** reattached to SAP- type B cells but not to type A cells. Recombinant 98-kDa type A **S-layer protein** and its truncated amino-terminal 65- and 50-kDa segments expressed in Escherichia coli retained the full and specific determinants for attachment. **S-layer protein** and purified homologous but not **heterologous** LPS in the presence of calcium produced insoluble complexes. By quantitative **enzyme**-linked immunosorbent assay, the **S-layer protein** copy number per C. fetus cell was determined to be approximately 105. In conclusion, C. fetus cells are encapsulated by a large number of **S -layer protein** molecules which may be specifically attached through the N-terminal half of the molecule to LPS in the presence of divalent cations.

L34 ANSWER 18 OF 18 EMBASE COPYRIGHT 2000 ELSEVIER SCI. B.V.DUPLICATE 9
AN 92152435 EMBASE
DN 1992152435
TI **Antigenic** diversity of the S-layer proteins from pathogenic strains of Aeromonas hydrophila and Aeromonas veronii biotype sobria.
AU Kostrzynska M.; Dooley J.S.G.; Shimojo T.; Sakata T.; Trust T.J.
CS Biochemistry/Microbiol. Dept., University of Victoria,Victoria, BC, Canada
SO Journal of Bacteriology, (1992) 174/1 (40-47).
ISSN: 0021-9193 CODEN: JOBAAY
CY United States
DT Journal; Article
FS 004 Microbiology
LA English
SL English
AB The **antigenic** relatedness of paracrystalline surface array proteins with subunit molecular weights of approximately 52,000 from isolates of Aeromonas hydrophila and Aeromonas veronii biotype sobria belonging to a single heat- stable serogroup was examined. **Enzyme** -linked immunosorbent assay and immunoblotting with two different polyclonal antisera against surface exposed and non-surface-exposed epitopes of the **S-layer protein** from A. hydrophila TF7 showed that the S-layer proteins of the mesophilic aeromonads were antigenically diverse. NH2-terminal amino acid sequence analysis of four antigenically different proteins showed that while the proteins were structurally related, they differed in primary sequence. Absorption experiments with **heterologous** live cells showed that cross-reactive epitopes were in non-surface-exposed regions of the S-layer proteins, while absorption with homologous live cells showed that the immunodominant epitopes of the **S- layer protein** of strain TF7 were strain specific and exposed on the surface of the native, tetragonal array produced by this strain. Proteolytic digestion of the TF7 **S-layer protein** with trypsin, chymotrypsin, or endoproteinase Glu-C produced an amino-terminal peptide of approximate M(r) 38,000 which was refractile to further proteolytic cleavage under nondenaturing conditions. This peptide carried the immunodominant surface- exposed region of the protein, and chemical cleavage with cyanogen bromide further mapped the

portion of these surface-exposed epitopes to a peptide of approximate M(r)
26,000, part of which maps within the M(r) 38,000 protease- resistant NH2-terminal peptide.

=> d his

(FILE 'HOME' ENTERED AT 16:15:00 ON 11 DEC 2000)

FILE 'EMBASE, MEDLINE, BIOSIS, BIOTECHDS, USPATFULL, CAPLUS, AGRICOLA, LIFESCI, CONFSCI' ENTERED AT 16:15:49 ON 11 DEC 2000

```
                E LUBITZ WERNER/AU
L1          692 S E1-E3
L2            0 S L1 AND FUSION PROTEIN (5A) BACILLUS
L3           26 S L1 AND (FUSION PROTEIN OR HETEROLOGOUS PROTEIN)
L4           12 DUP REM L3 (14 DUPLICATES REMOVED)
L5           36 S L1 AND S LAYER PROTEIN
L6           14 DUP REM L5 (22 DUPLICATES REMOVED)
                E SLEYTR UWE/AU
L7          735 S E1 OR E3 OR E4 OR E5
L8           15 S L7 AND (FUSION PROTEIN OR HETEROLOGOUS)
L9            4 DUP REM L8 (11 DUPLICATES REMOVED)
L10         213 S L7 AND S LAYER PROTEIN
L11           6 S L10 AND (FUSION PROTEIN OR HETEROLOGOUS)
                E KUEN BEATRIX/AU
L12          60 S E1-E3
L13           5 S L12 AND (FUSION PROTEIN OR HETEROLOGOUS)
L14          41 S L12 AND S LAYER PROTEIN
L15          14 DUP REM L14 (27 DUPLICATES REMOVED)
                E TRUPPE MICHAELA/AU
L16           9 S E1-E3
L17           8 DUP REM L16 (1 DUPLICATE REMOVED)
                E HOWORKA STEFAN/AU
L18          24 S E1 OR E3 OR E4
L19          13 S L18 AND S LAYER PROTEIN
L20           4 DUP REM L19 (9 DUPLICATES REMOVED)
                E RESCH STEPHANKA/AU
L21           6 S E1 OR E2 OR E4
L22           4 DUP REM L21 (2 DUPLICATES REMOVED)
                E SCHROLL GERHARD/AU
L23           8 S E3
L24           5 DUP REM L23 (3 DUPLICATES REMOVED)
                E SARA MARGIT/AU
L25         119 S E3
L26          70 S L25 AND S LAYER PROTEIN
L27           0 S L26 AND FUSION PROTEIN
L28         916 S S LAYER PROTEIN
L29         345 S SURFACE LAYER PROTEIN
L30          24 S L29 AND (FUSION PROTEIN OR HETEROLOGOUS)
L31          16 DUP REM L30 (8 DUPLICATES REMOVED)
L32          97 S L28 AND (FUSION PROTEIN OR HETEROLOGOUS)
L33          37 S L32 AND (IMMUNOGENIC OR EPITOPE OR ENZYME? OR CYTOKINE OR AN
L34          18 DUP REM L33 (19 DUPLICATES REMOVED)
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=> s l28 and (streptavidin or herpes virus or bacterial luciferase or interleukin or interferon or protein G or protein A)

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   4 FILES SEARCHED...
   5 FILES SEARCHED...
L35          68 L28 AND (STREPTAVIDIN OR HERPES VIRUS OR BACTERIAL LUCIFERASE
                OR INTERLEUKIN OR INTERFERON OR PROTEIN G OR PROTEIN A)
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=> dup rem 135

PROCESSING COMPLETED FOR L35
L36 37 DUP REM L35 (31 DUPLICATES REMOVED)

=> d bib ab 1-37

L36 ANSWER 1 OF 37 BIOSIS COPYRIGHT 2000 BIOSIS
AN 2000:504103 BIOSIS
DN PREV200000504103
TI Two-dimensional gel electrophoresis analyses of pH-dependent protein expression in facultatively alkaliphilic Bacillus pseudofirmus OF4 lead to characterization of an **S-layer protein** with a role in alkaliphily.
AU Gilmour, Raymond; Messner, Paul; Guffanti, Arthur A.; Kent, Rebecca; Scheberl, Andrea; Kendrick, Nancy; Krulwich, Terry Ann (1)
CS (1) Department of Biochemistry and Molecular Biology, Mount Sinai School of Medicine, 1 Gustave L. Levy Place, New York, NY, 10029 USA
SO Journal of Bacteriology, (November, 2000) Vol. 182, No. 21, pp. 5969-5981.
print.
ISSN: 0021-9193.
DT Article
LA English
SL English
AB The large majority of proteins of alkaliphilic Bacillus pseudofirmus OF4 grown at pH 7.5 and 10.5, as studied by two-dimensional gel electrophoresis analyses, did not exhibit significant pH-dependent variation. A new surface layer protein (SlpA) was identified in these studies. Although the prominence of some apparent breakdown products of SlpA in gels from pH 10.5-grown cells led to discovery of the alkaliphile S-layer, the largest and major SlpA forms were present in large amounts in gels from pH 7.5-grown cells as well. slpA RNA abundance was, moreover, unchanged by growth pH. SlpA was similar in size to homologues from nonalkaliphiles but contained fewer Arg and Lys residues. An slpA mutant strain (RG21) lacked an exterior S-layer that was identified in the wild type by electron microscopy. Electrophoretic analysis of whole-cell extracts further indicated the absence of a 90-kDa band in the mutant. This band was prominent in wild-type extracts from both pH 7.5- and 10.5-grown cells. The wild type grew with a shorter lag phase than RG21 at either pH 10.5 or 11 and under either Na+-replete or suboptimal Na+ concentrations. The extent of the adaptation deficit increased with pH elevation and suboptimal Na+. By contrast, the mutant grew with a shorter lag and faster growth rate than the wild type at pH 7.5 under Na+-replete and suboptimal Na+ conditions, respectively. Logarithmically growing cells of the two strains exhibited no significant differences in growth rate, cytoplasmic pH regulation, starch utilization, motility, Na+-dependent transport of alpha-aminoisobutyric acid, or H+-dependent synthesis of ATP. However, the capacity for Na+-dependent pH homeostasis was diminished in RG21 upon a sudden upward shift of external pH from 8.5 to 10.5. The energy cost of retaining the SlpA layer at near-neutral pH is apparently adverse, but the constitutive presence of SlpA enhances the capacity of the extremophile to adjust to high pH.

L36 ANSWER 2 OF 37 CAPLUS COPYRIGHT 2000 ACS
AN 2000:476644 CAPLUS
DN 133:345267
TI Gene cloning and expression and secretion of Listeria monocytogenes bacteriophage-lytic enzymes in Lactococcus lactis
AU Gaeng, Susanne; Scherer, Siegfried; Neve, Horst; Loessner, Martin J.

CS Institut fur Mikrobiologie, FML Weihenstephan, Institut fur Mikrobiologie,
FML Weihenstephan, Technische Universitat Munchen, Freising, D-85350, Germany
SO Appl. Environ. Microbiol. (2000), 66(7), 2951-2958
CODEN: AEMIDF; ISSN: 0099-2240
PB American Society for Microbiology
DT Journal
LA English
AB Bacteriophage lysins (Ply), or endolysins, are phage-encoded cell wall lytic enzymes which are synthesized late during virus multiplication and mediate the release of progeny virions. Bacteriophages of the pathogen Listeria monocytogenes encode endolysin enzymes which specifically hydrolyze the crosslinking peptide bridges in Listeria peptidoglycan. Ply118 is a 30.8-kDa L-alanyl-D-glutamate peptidase and Ply511 (36.5 kDa) acts as N-acetylmuramoyl-L-alanine amidase. In order to establish dairy starter cultures with biopreservation properties against L. monocytogenes contaminations, we have introduced ply118 and ply511 into Lactococcus lactis MG1363 by using a pTRKH2 backbone. The genes were expressed under control of the lactococcal promoter P32, which proved superior to other promoters (P21 and P59) tested in this study. High levels of active enzymes were produced and accumulated in the cytoplasmic cell fractions but were not released from the cells at significant levels. Therefore, ply511 was genetically fused with the SPslpA nucleotide sequence encoding the Lactobacillus brevis **S-layer protein**
signal peptide. Expression of SPslpA-ply511 from pSL-PL511 resulted in secretion of functional Ply511 enzyme from L. lactis cells. One clone expressed an unusually strong lytic activity, which was found to be due to
a 115-bp deletion that occurred within the 3'-end coding sequence of SPslpA-ply511, which caused a frameshift mutation and generated a stop codon. Surprisingly, the resulting carboxy-terminal deletion of 80 amino acids in the truncated Ply511.DELTA.(S262-K341) mutant polypeptide strongly increased its lytic activity. Proteolytic processing of the secretion competent SPSlpA-Ply511 propeptide following membrane translocation had no influence on enzyme activity. Immunoblotting expts. using both cytoplasmic and supernatant fractions indicated that the enzyme
was quant. exported from the cells and secreted into the surrounding medium, where it caused rapid lysis of L. monocytogenes cells. Moreover, transformation of pSL-PL511.DELTA.C into L. lactis Bu2-129, a lactose-utilizing strain that can be employed for fermn. of milk, also resulted in secretion of functional enzyme and showed that the vector is compatible with the native lactococcal plasmids.
RE.CNT 47
RE
(3) Cardinal, M; Food Biotechnol 1997, V11, P129 CAPLUS
(4) Cintas, L; Appl Environ Microbiol 1995, V61, P2643 CAPLUS
(5) Dickely, F; Mol Microbiol 1995, V15, P839 CAPLUS
(7) Dower, W; Nucleic Acids Res 1988, V16, P6127 CAPLUS
(9) Foegeding, P; Appl Environ Microbiol 1992, V58, P884 CAPLUS
ALL CITATIONS AVAILABLE IN THE RE FORMAT

L36 ANSWER 3 OF 37 EMBASE COPYRIGHT 2000 ELSEVIER SCI. B.V.DUPLICATE 1
AN 2000073429 EMBASE
TI Use of defined mutants to assess the role of the Campylobacter rectus S-layer in bacterium-epithelial cell interactions.
AU Wang B.; Kraig E.; Kolodrubetz D.
CS D. Kolodrubetz, Department of Microbiology, Univ. of Texas Health Science Center, 7703 Floyd Curl Dr., San Antonio, TX 78229, United States.
kolodrubetz@uthsesa.edu
SO Infection and Immunity, (2000) 68/3 (1465-1473).
Refs: 49
ISSN: 0019-9567 CODEN: INFIBR
CY United States

DT Journal; Article
FS 004 Microbiology
LA English
SL English
AB Campylobacter rectus is a periodontal pathogen with a 150-kDa protein on its cell surface. This protein forms a paracrystalline lattice, called the S- layer, surrounding the outer membrane of this gram-negative bacterium. To initiate a genetic analysis of the possible role of the S-layer in the initial interaction of C. rectus with host epithelial cells, C. rectus strains lacking the **S-layer protein** gene (crsA) were constructed by allelic exchange mutagenesis. Surprisingly, the lack of the S-layer had only a minor effect on the interaction of C. rectus with HEp-2 epithelial cells; CrsA+ cells were 30 to 50% more adherent than were CrsA- bacteria. Since the host cell expression of cytokines appears to play an important role in the pathogenesis of periodontal diseases, the effect of the S-layer on the epithelial cell cytokine response was also examined by quantitative reverse transcriptase PCR and enzyme-linked immunosorbent assay. Although there were no changes in the mRNA levels for the anti-inflammatory cytokines **interleukin** -1 receptor agonist (IL-1ra), IL-13, and transforming growth factor .beta., the expression and secretion of the proinflammatory cytokines IL-6, IL-8, and tumor necrosis factor alpha (TNF-.alpha.) were significantly induced by both wild-type C. rectus and CrsA- bacteria. Interestingly, the kinetics of cytokine induction differed for the CrsA+ and CrsA- bacteria. At early time points, the HEp-2 cells challenged with CrsA- bacteria produced higher levels of IL-6, IL-8, and TNF-.alpha. mRNA and protein than did cells challenged with CrsA+ bacteria. We conclude that C. rectus may help initiate periodontitis by increasing the expression of proinflammatory cytokines and that the S-layer may temper this response to facilitate the survival of C. rectus at the site of infection.

L36 ANSWER 4 OF 37 EMBASE COPYRIGHT 2000 ELSEVIER SCI. B.V.DUPLICATE 2
AN 2000013805 EMBASE
TI S-layer-coated liposomes as a versatile system for entrapping and binding target molecules.
AU Mader C.; Kupcu S.; Sleytr U.B.; Sara M.
CS M. Sara, Zentrum fur Ultrastrukturforschung, Ludwig Boltzmann-Institut, Universitat fur Bodenkultur Wien, Gregor-Mendelstr. 33, A-1180 Vienna, Austria. sara@edvl.boku.ac.at
SO Biochimica et Biophysica Acta - Biomembranes, (2000) 1463/1 (142-150). Refs: 33
ISSN: 0005-2736 CODEN: BBBMBS
PUI S 0005-2736(99)00190-X
CY Netherlands
DT Journal; Article
FS 029 Clinical Biochemistry
004 Microbiology
LA English
SL English
AB In the present study, unilamellar liposomes coated with the crystalline bacterial cell surface layer (**S-layer**) **protein** of Bacillus stearothermophilus PV72/p2 were used as matrix for defined binding of functional molecules via the avidin- or **streptavidin** -biotin bridge. The liposomes were composed of dipalmitoyl phosphatidylcholine, cholesterol and hexadecylamine in a molar ratio of 10:5:4 and they had an average size of 180 nm. For introducing specific functions into the S-layer lattice without affecting substances encapsulated within the liposomes, crosslinking and activation reagents had to be identified which did not penetrate the liposomal membrane. Among different reagents, a hydrophilic dialdehyde generated by periodate

cleavage of raffinose and a sulfo-succinimide activated dicarboxylic acid were found to be impermeable for the liposomal membrane. Both reagents completely crosslinked the S-layer lattice without interfering with its regular structure. Biotinylation of S-layer-coated liposomes was achieved by coupling p-diazobenzoyl biocytin which preferably reacts with the phenolic residue of tyrosine or with the imidazole ring of histidine. By applying this method, two biotin residues accessible for subsequent avidin binding were introduced per S-layer subunit. As visualized by labeling with biotinylated ferritin, an ordered monomolecular layer of **streptavidin** was formed on the surface of the S-layer-coated liposomes. As a second model system, biotinylated anti-human IgG was attached via the **streptavidin** bridge to the biotinylated S-layer-coated liposomes. The biological activity of the bound anti-human IgG was confirmed by ELISA. Copyright (C) 2000 Elsevier Science B.V.

L36 ANSWER 5 OF 37 USPATFULL
AN 1999:137013 USPATFULL
TI Expression and secretion of heterologous polypeptides from caulobacter
IN Smit, John, Richmond, Canada
Bingle, Wade H., Vancouver, Canada
Nomellini, John F., Richmond, Canada
PA The University of British Columbia, Canada (non-U.S. corporation)
PI US 5976864 19991102
AI US 1996-614377 19960312 (8)
RLI Continuation-in-part of Ser. No. US 1994-194290, filed on 9 Feb 1994, now patented, Pat. No. US 5500353 which is a continuation-in-part of Ser. No. US 1992-895367, filed on 9 Jun 1992, now abandoned
DT Utility
EXNAM Primary Examiner: Wax, Robert A.; Assistant Examiner: Nashed, Nashaat T.
LREP Fish & Richardson P.C.
CLMN Number of Claims: 14
ECL Exemplary Claim: 2
DRWN 14 Drawing Figure(s); 13 Drawing Page(s)
LN.CNT 1609
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
AB DNA constructs are provided which code for at least the extreme C-terminal amino acids of the rsaA protein of Caulobacter crescentus fused with heterologous polypeptides. Baterial cells containing, or which express the DNA constructs and secrete the resulting protein are also provided. Chimeric proteins including the C-terminal amino acids of the rsaA protein are provided, including chimeric proteins comprising antigenic epitopes of the Infectious Hematopoietic Necrosis Virus.

L36 ANSWER 6 OF 37 USPATFULL
AN 1999:24489 USPATFULL
TI Expression of surface layer proteins
IN Deblaere, Rolf Y., Waarschoot, Belgium
Desomer, Jan, Drongen, Belgium
Dhaese, Patrick, Drongen, Belgium
PA Solvay (Societe Anonyme), Brussels, Belgium (non-U.S. corporation)
PI US 5874267 19990223
WO 9519371 19950720
AI US 1996-682517 19960917 (8)
WO 1995-EP147 19950113
19960917 PCT 371 date
19960917 PCT 102(e) date
PRAI GB 1994-650 19940114
DT Utility
EXNAM Primary Examiner: Hutzell, Paula K.; Assistant Examiner: Bakalyar, Heather A.
LREP McDermott, Will & Emery
CLMN Number of Claims: 1

ECL Exemplary Claim: 1
DRWN 41 Drawing Figure(s); 37 Drawing Page(s)
LN.CNT 2742
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
AB A host cell which is provided with a S-layer comprising a fusion polypeptide consisting essentially of:

(a) at least sufficient of a **S-layer protein** for a S-layer composed thereof to assemble, and

(b) a heterologous polypeptide which is fused to either the carboxy terminus of (a) or the amino terminus of (a) and which is thereby presented on the outer surface of the said cell; can be used as a vaccine, for screening for proteins and antigens and as a support for immobilizing an enzyme, peptide or antigen. A process of transforming B.

Sphaericus cells comprising electroporation is also provided.

L36 ANSWER 7 OF 37 CAPLUS COPYRIGHT 2000 ACS
AN 1999:96508 CAPLUS
DN 130:178339
TI Production of genetically engineered **S-layer protein** that is secreted into the periplasm or extracellularly and that can contain integrated proteins for affinity and immuno reactions
IN Lubitz, Werner; Resch, Stephanie
PA Austria
SO Ger. Offen., 34 pp.
CODEN: GWXXBX
DT Patent
LA German
FAN.CNT 1

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
PI DE 19732829	A1	19990204	DE 1997-19732829	19970730
WO 9906567	A1	19990211	WO 1998-EP4723	19980727

W: AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GE, GH, GM, HR, HU, ID, IL, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, UA, UG, US, UZ, VN, YU, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM
RW: GH, GM, KE, LS, MW, SD, SZ, UG, ZW, AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG

AU 9890705	A1	19990222	AU 1998-90705	19980727
EP 1005553	A1	20000607	EP 1998-942648	19980727

R: AT, BE, CH, DE, DK, ES, FR, GB, IT, LI, NL, SE, IE

PRAI DE 1997-19732829 19970730
WO 1998-EP4723 19980727
AB The invention concerns the prodn. of recombinant **S-layer protein** expressed in Gram-neg. prokaryote or eukaryote host cells using the sbsA and sbsB clones of the Bacillus stearothermophilus PV72, that code for the **S-layer protein** and the prokaryote signal peptide; the vector also contains inserts at convenient sites that code for various peptides, e.g. cysteine residues, DNA-binding epitopes, metal-binding epitopes, allergens, antigens, **streptavidin**, enzymes etc. In case the fusion protein is expressed in eukaryotes, the vector includes sequences coding for eukaryote signal peptides. The host cell contains at least two types of genes that code for the a non-modified **S-layer protein** and for a modified **S-layer protein** that is fused with a peptide used biochem. reactions. E.coli is a typical host cell.

L36 ANSWER 8 OF 37 MEDLINE
AN 1999214094 MEDLINE

DN 99214094
TI Distinct affinity of binding sites for S-layer homologous domains in Clostridium thermocellum and Bacillus anthracis cell envelopes.
AU Chauvaux S; Matuschek M; Beguin P
CS Unite de Physiologie Cellulaire, Departement des Biotechnologies, Institut Pasteur, 75724 Paris Cedex 15, France.. chauvaux@pasteur.fr
SO JOURNAL OF BACTERIOLOGY, (1999 Apr) 181 (8) 2455-8. Journal code: HH3. ISSN: 0021-9193.
CY United States
DT Journal; Article; (JOURNAL ARTICLE)
LA English
FS Priority Journals
EM 199907
EW 19990703
AB Binding parameters were determined for the SLH (S-layer homologous) domains from the Clostridium thermocellum outer layer protein OlpB, from the C. thermocellum **S-layer protein** SlpA, and from the Bacillus anthracis S-layer proteins EA1 and Sap, using cell walls from C. thermocellum and B. anthracis. Each SLH domain bound to C. thermocellum and B. anthracis cell walls with a different KD, ranging between 7.1 x 10(-7) and 1.8 x 10(-8) M. Cell wall binding sites for SLH domains displayed different binding specificities in C. thermocellum and B. anthracis. SLH-binding sites were not detected in cell walls of Bacillus subtilis. Cell walls of C. thermocellum lost their affinity for SLH domains after treatment with 48% hydrofluoric acid but not after treatment with formamide or dilute acid. A soluble component, extracted from C. thermocellum cells by sodium dodecyl sulfate treatment, bound the SLH domains from C. thermocellum but not those from B. anthracis proteins. A corresponding component was not found in B. anthracis.

L36 ANSWER 9 OF 37 MEDLINE
AN 1999444136 MEDLINE
DN 99444136
TI The effect of **S-layer protein** adsorption and crystallization on the collective motion of a planar lipid bilayer studied by dynamic light scattering.
AU Hirn R; Schuster B; Sleytr U B; Bayerl T M
CS Universitat Wurzburg, Physikalisches Institut EP-5, 97074 Wurzburg, Germany.
SO BIOPHYSICAL JOURNAL, (1999 Oct) 77 (4) 2066-74. Journal code: A5S. ISSN: 0006-3495.
CY United States
DT Journal; Article; (JOURNAL ARTICLE)
LA English
FS Priority Journals
EM 200001
EW 20000104
AB A dedicated dynamic light scattering (DLS) setup was employed to study the undulations of freely suspended planar lipid bilayers, the so-called black lipid membranes (BLM), over a previously inaccessible spread of frequencies (relaxation times ranging from 10(-2) to 10(-6) s) and wavevectors (250 cm(-1) < q < 38,000 cm(-1)). For a BLM consisting of 1,2-dielaidoyl-sn-3-glycero-phosphocholine (DEPC) doped with two different proportions of the cationic lipid analog dioctadecyl-dimethylammonium bromide (DODAB) we observed an increase of the lateral tension of the membrane with the DODAB concentration. The experimentally determined dispersion behavior of the transverse shear mode was in excellent agreement with the theoretical predictions of a first-order hydrodynamic theory. The symmetric adsorption of the crystalline bacterial cell surface

layer (S-layer) proteins from Bacillus coagulans E38-66 to a weakly cationic BLM (1.5 mol % DODAB) causes a drastic reduction of the membrane tension well beyond the previous DODAB-induced tension increase. The likely reason for this behavior is an increase of molecular order along the lipid chains by the protein and/or partial protein penetration into the lipid headgroup region. **S-layer protein** adsorption to a highly cationic BLM (14 mol % DODAB) shows after 7 h incubation time an even stronger decrease of the membrane tension by a factor of five, but additionally a significant increase of the (previously negligible) surface viscosity, again in excellent agreement with the hydrodynamic theory. Further incubation (24 h) shows a drastic increase of the membrane bending energy by three orders of magnitude as a result of a large-scale, two-dimensional recrystallization of the S-layer proteins at both sides of the BLM. The results demonstrate the potential of the method for the assessment of the different stages of protein adsorption and recrystallization at a membrane surface by measurements of the collective membrane modes and their analysis in terms of a hydrodynamic theory.

L36 ANSWER 10 OF 37 MEDLINE DUPLICATE 3
AN 1999177548 MEDLINE
DN 99177548
TI The expression signals of the Lactobacillus brevis slpA gene direct efficient heterologous protein production in lactic acid bacteria.
AU Kahala M; Palva A
CS Agricultural Research Centre of Finland, Food Research Institute, Finland.
SO APPLIED MICROBIOLOGY AND BIOTECHNOLOGY, (1999 Jan) 51 (1) 71-8. Journal code: AMC. ISSN: 0175-7598.
CY GERMANY: Germany, Federal Republic of
DT Journal; Article; (JOURNAL ARTICLE)
LA English
FS Priority Journals
EM 199906
AB A cassette based on the expression signals of the Lactobacillus brevis surface (**S**)**-layer protein** gene (slpA) was constructed. The low-copy-number vector pKTH2095, derived from pGK12, was used as the cloning vector. The efficiency of slpA promoters in intracellular protein production was studied using three reporter genes, beta-glucuronidase (gusA), luciferase (luc) and aminopeptidase N (pepN) in three different lactic acid bacteria hosts: Lactococcus lactis, Lactobacillus plantarum and Lactobacillus gasseri. The S-layer promoters were recognized in each strain and especially L. lactis and Lb. plantarum exhibited high levels of transcripts. The production kinetics of reporter proteins was studied as a function of growth. The GusA, Luc and PepN activities varied considerably among the lactic acid bacterial strains studied. The highest levels of beta-glucuronidase and luciferase activity were obtained in L. lactis. The level of GusA obtained in L. lactis corresponded to over 15% of the total cellular proteins. The highest level of aminopeptidase N activity was achieved in Lb. plantarum where PepN corresponded up to 28% of the total cellular proteins at the late exponential phase of growth. This level of PepN activity is 30-fold higher than that in Lb. helveticus, which is the species from which the pepN gene originates.

L36 ANSWER 11 OF 37 USPATFULL
AN 1998:85822 USPATFULL
TI Gene and protein applicable to the preparation of vaccines for rickettsia prowazekii and rickettsia typhi and the detection of both

IN Carl, Mitchell, San Diego, CA, United States
Dobson, Michael E., Rockville, MD, United States
Ching, Wei-Mei, Bethesda, MD, United States
Dasch, Gregory A., Wheaton, MD, United States
PA The United States of America as represented by the Secretary of the Navy, Washington, DC, United States (U.S. government)
PI US 5783441 19980721
AI US 1993-169927 19931220 (8)
RLI Continuation-in-part of Ser. No. US 1991-742128, filed on 9 Aug 1991, now abandoned
DT Utility
EXNAM Primary Examiner: Housel, James C.; Assistant Examiner: Shaver, Jennifer
LREP Spevack, A. David; Garvert, William C.
CLMN Number of Claims: 2
ECL Exemplary Claim: 1
DRWN 5 Drawing Figure(s); 3 Drawing Page(s)
LN.CNT 928
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
AB All or part of the DNA sequence of the gene which encodes the S-**layer protein** of R. prowazekii as illustrated in Sequence ID No. 1 as well as a truncated identical piece of this gene in R. typhi as well as the 5' and 3' noncoding regions can be used for vaccination against typhus and spotted fever rickettsial infection or to diagnose the diseases caused by these bacteria. The invention is also accomplished by the deduced amino acid sequence of the **S-layer protein** of R. prowazekii derived from the DNA sequence of the encoding gene. Further, the invention includes the peptide or protein products based on all or parts of this gene.

L36 ANSWER 12 OF 37 MEDLINE
AN 1998129094 MEDLINE
DN 98129094
TI Identification of a region responsible for binding to the cell wall within the **S-layer protein** of Clostridium thermocellum.
AU Lemaire M; Miras I; Gounon P; Beguin P
CS Unite de Physiologie Cellulaire, Institut Pasteur, Paris, France.
SO MICROBIOLOGY, (1998 Jan) 144 (Pt 1) 211-7.
Journal code: BXW. ISSN: 1350-0872.
CY ENGLAND: United Kingdom
DT Journal; Article; (JOURNAL ARTICLE)
LA English
FS Priority Journals
OS GENBANK-U79117
EM 199806
EW 19980602
AB The protomer forming the S-layer of Clostridium thermocellum was identified as a 140 kDa protein which was non-covalently bound to the cell wall. Cloning and sequencing of the corresponding gene revealed an open reading frame of 3108 nucleotides encoding a polypeptide of 1036 amino acids, termed SlpA. The amino acid composition of SlpA matches the composition of a previously described exocellular glycoprotein. SlpA shared extensive similarity with the **S-layer protein** of Bacillus sphaericus and with the outer wall protein of Bacillus brevis. In addition, the amino-terminal region of SlpA contained a segment presenting similarities with segments termed SLH (S-layer homologous), which are found in several bacterial exoproteins. A polypeptide of 209 residues comprising this segment was shown to bind to cell walls extracted from C. thermocellum cells.

L36 ANSWER 13 OF 37 BIOTECHDS COPYRIGHT 2000 DERWENT INFORMATION LTD
AN 1997-11103 BIOTECHDS
TI Preparation of S-layer proteins by expressing sbs-A gene in Gram-negative bacterium;
for use as e.g. vaccine or adjuvant
AU Lubitz W; Sleytr U; Kuen B
PA Lubitz W; Sleytr U
LO Vienna, Austria.
PI DE 19603649 7 Aug 1997
AI DE 1996-1003649 1 Feb 1996
PRAI DE 1996-1003649 1 Feb 1996
DT Patent
LA German
OS WPI: 1997-394558 [37]
AB A new method for the preparation of **S-layer protein** (I) involves transforming a Gram-negative prokaryote, preferably Escherichia coli, with a nucleic acid encoding (I) contained on a vector, and culturing the transformed cells. The nucleic acid may contain one or more inserts, preferably encoding Cys residues, regions with many charged amino acids or Tyr, DNA-or metal-binding epitopes, immune, allergenic or antigenic epitopes, **streptavidin**, enzymes or cytokine- or antibody-binding proteins. (I) is useful as a recombinant vaccine or adjuvant, especially when combined with a bacterial ghost that may contain additional epitopes in its membrane. Other uses, depending on the inserted protein, include (a) universal adjuvant for biotinylated reactants for immunological or hybridization assays, (b) induction of immune responses, (c) reagent for removing cytokine or toxin from serum, (d) molecular spinning nozzle and (e) molecular laser. When expressed in Gram-negative cells, (I) is produced in the form of monomolecular layers rather than as inclusion bodies as in Gram-positive bacteria. (31pp)

L36 ANSWER 14 OF 37 USPATFULL
AN 97:68577 USPATFULL
TI Processes for the synthesis of sialyl Lewis.sup.x compounds
IN Srivastava, Om, Edmonton, Canada
Gregson, Jonathan M., Edmonton, Canada
PA Glycomed Incorporated, Alameda, CA, United States (U.S. corporation)
PI US 5654412 19970805
AI US 1996-657456 19960529 (8)
DT Utility
EXNAM Primary Examiner: Fonda, Kathleen K.
LREP Burns, Doane, Swecker & Mathis, L.L.P.
CLMN Number of Claims: 12
ECL Exemplary Claim: 1
DRWN 3 Drawing Figure(s); 3 Drawing Page(s)
LN.CNT 930
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
AB Disclosed are processes for the chemical synthesis of sialyl Lewis.sup.x
-Y compounds where Y is --OH, --NHR, --SH, --SR or --OR, and R is an aglycon of at least one carbon atom.

L36 ANSWER 15 OF 37 USPATFULL
AN 97:59181 USPATFULL
TI Time dependent administration of oligosaccharide glycosides related to blood group determinants having a type I or type II core structure in reducing inflammation in a sensitized mammal arising form exposure to an antigen
IN Ippolito, Robert M., Edmonton, Canada
Haque, Wasimul, Edmonton, Canada
Jiang, Cong, San Diego, CA, United States

Hanna, H. Rizk, Edmonton, Canada
Venot, Andre P., Agoura Hills, CA, United States
Nikrad, Pandurang V., Edmonton, Canada
Kashem, Mohammed A., Thousand Oaks, CA, United States
Smith, Richard, Edmonton, Canada
Srivastava, Om P., Jackson Heights, Canada

PA Alberta Research Council, Alberta, Canada (non-U.S. corporation)
PI US 5646123 19970708
AI US 1995-405785 19950317 (8)
RLI Continuation of Ser. No. US 1993-81214, filed on 25 Jun 1993, now abandoned which is a continuation of Ser. No. US 1992-988518, filed on 10 Dec 1992, now abandoned which is a continuation-in-part of Ser. No. US 1992-895930, filed on 9 Jun 1992, now abandoned which is a continuation-in-part of Ser. No. US 1992-889017, filed on 26 May 1992, now abandoned which is a continuation-in-part of Ser. No. US 1991-714161, filed on 10 Jun 1991, now abandoned
DT Utility
EXNAM Primary Examiner: Fonda, Kathleen K.
LREP Burns, Doane, Swecker & Mathis, LLP
CLMN Number of Claims: 4
ECL Exemplary Claim: 1
DRWN 66 Drawing Figure(s); 63 Drawing Page(s)
LN.CNT 6831
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
AB Disclosed are methods for reducing the degree of antigen induced inflammation in a sensitized mammals. The disclosed methods employ oligosaccharide glycosides related to blood group determinants having a type I or type II core structure wherein the administration of such oligosaccharide glycosides is after initiation of the mammal's immune response but at or prior one-half the period of time required to effect maximal antigen-induced inflammation.

L36 ANSWER 16 OF 37 CAPLUS COPYRIGHT 2000 ACS
AN 1997:536912 CAPLUS
DN 127:201021
TI Expression of S-layer proteins in Gram-negative bacteria and recombinant chimeric S-layer proteins for use as vaccines
IN Lubitz, Werner; Sleytr, Uwe; Kuen, Beatrix; Truppe, Michaela; Howorka, Stefan; Resch, Stepanka; Schroll, Gerhard; Sara, Margit
PA Lubitz, Werner, Austria; Sleytr, Uwe; Kuen, Beatrix; Truppe, Michaela; Howorka, Stefan; Resch, Stepanka; Schroll, Gerhard; Sara, Margit
SO PCT Int. Appl., 65 pp.
CODEN: PIXXD2
DT Patent
LA German
FAN.CNT 1

	PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
PI	WO 9728263	A1	19970807	WO 1997-EP432	19970131
	W: AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GE, HU, IL, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, TJ, TM, TR, TT, UA, UG, US, UZ, VN, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM				
	RW: KE, LS, MW, SD, SZ, UG, AT, BE, CH, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, BF, BJ, CF, CG, CI, CM, GA, GN, ML, MR, NE, SN, TD, TG				
	DE 19603649	A1	19970807	DE 1996-19603649	19960201
	CA 2245584	AA	19970807	CA 1997-2245584	19970131
	AU 9717203	A1	19970822	AU 1997-17203	19970131
	AU 713999	B2	19991216		
	EP 882129	A1	19981209	EP 1997-904360	19970131
	R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, SE, MC, PT, IE, FI				
	CN 1213402	A	19990407	CN 1997-192940	19970131

JP 2000503850 T2 20000404 JP 1997-527307 19970131
PRAI DE 1996-19603649 19960201
WO 1997-EP432 19970131
AB The invention concerns processess for the recombinant prepn. of S-layer proteins in Gram-neg. host cells. In addn., the nucleotide sequence of a new S-layer gene, the sbsB gene of Bacillus stearothermophilus, and a process for prepn. of modified S-layer proteins is disclosed.
Recombinant
Escherichia coli expressing the sbsA gene of B. stearothermophilus and chimeric sbsA genes encoding SbsA into which various peptides, proteins and enzymes have been inserted were prepd. and cultured to produce the proteins.

L36 ANSWER 17 OF 37 BIOSIS COPYRIGHT 2000 BIOSIS
AN 1998:87452 BIOSIS
DN PREV199800087452
TI Bet v 1, the major birch pollen allergen, conjugated to crystalline bacterial cell surface proteins, expands allergen-specific T cells of the Th1/Th0 phenotype in vitro by induction of IL-12.
AU Jahn-Schmid, Beatrice; Siemann, Ute; Zenker, Andrea; Bohle, Barbara; Messner, Paul; Unger, Frank M.; Sleytr, Uwe B.; Scheiner, Otto; Kraft, Dietrich; Ebner, Christof (1)
CS (1) Inst. Allgemeine Experimentelle Pathologie, Univ. Wien, AKH-EWB-OST 3Q, Waehringer Guertel 18-20, 1090 Wien Austria
SO International Immunology, (Dec., 1997) Vol. 9, No. 12, pp. 1867-1874. ISSN: 0953-8178.
DT Article
LA English
AB Modulation of allergic immune responses by using adequate adjuvants is a promising concept for future immunotherapy of type I hypersensitivity. In the present study, recombinant Bet v 1 (rBet v 1, the major birch pollen allergen) was conjugated to cross-linked crystalline surface layer proteins (SL) derived from Gram-positive eubacteria. T cell lines (TCL) and clones (TCC) were established from peripheral blood of birch pollen-allergic patients. TCL and TCC were induced either using rBet v 1 alone or rBet v 1/SL conjugates (rBet v 1/SL) as initial antigen
stimulus.
Cytokine production after re-stimulation with rBet v 1 was investigated. TCL initiated with rBet v 1/SL showed significantly increased IFN-gamma production as compared to rBet v 1-selected TCL. TCC were established
from
TCL of five patients. As expected, the majority of CD4+ TCC induced by rBet v 1 (55%) displayed a Th2-like pattern of cytokine production. However, only 21% of Bet v 1 -specific TCC isolated from TCL established with the Bet v 1/SL revealed this phenotype. The majority of SL-specific TCC (80%) belonged to the Th1 phenotype. In cultures of peripheral blood mononuclear cells, both, SL and Bet v 1/SL (but not rBet v 1) stimulated the production of high levels of IL-12, a pivotal mediator of Th1 responses. Moreover, stimulation of rBet v 1-induced TCC with rBet v 1/SL led to an Increased IFN-gamma production. This effect could be reversed
by
neutralizing anti-IL-12 mAb. Together these results indicate an adjuvant effect of SL mediated by IL-12. Our results indicate that bacterial components, such as SL, displaying adjuvant effects may be suitable for immunotherapeutical vaccines for type I allergy.

L36 ANSWER 18 OF 37 USPATFULL
AN 96:111448 USPATFULL
TI Immunosuppressive and tolerogenic modified Lewis.sup.x compounds
IN Ippolito, Robert M., Edmonton, Canada
Haque, Wasimul, Edmonton, Canada
Jiang, Cong, Edmonton, Canada
Hanna, H. Rizk, Edmonton, Canada
Venot, Andre P., Edmonton, Canada
Nikrad, Pandurang V., Edmonton, Canada

Kashem, Mohammed A., Edmonton, Canada
Smith, Richard H., Edmonton, Canada
PA Alberta Research Council, Canada (non-U.S. corporation)
PI US 5580858 19961203
AI US 1994-337461 19941104 (8)
RLI Continuation of Ser. No. US 1992-895930, filed on 9 Jun 1992, now abandoned which is a continuation-in-part of Ser. No. US 1992-889017, filed on 26 May 1992, now abandoned which is a continuation-in-part of Ser. No. US 1991-714161, filed on 10 Jun 1991
DT Utility
EXNAM Primary Examiner: Robinson, Douglas W.; Assistant Examiner: Fonda, Kathleen Kahler
LREP Burns, Doane, Swecker & Mathis
CLMN Number of Claims: 10
ECL Exemplary Claim: 1
DRWN 22 Drawing Figure(s); 21 Drawing Page(s)
LN.CNT 2960
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
AB Disclosed are novel Lewis.sup.x and Lewis.sup.a analogues, pharmaceutical compositions containing such analogues, methods for their preparation and methods for their use.

L36 ANSWER 19 OF 37 USPATFULL
AN 96:77808 USPATFULL
TI Methods for the synthesis of monofucosylated oligosaccharides terminating in di-N-acetyllactosaminyl structures
IN Kashem, Mohammed A., Edmonton, Canada
Venot, Andre P., Edmonton, Canada
Smith, Richard, Edmonton, Canada
PA Alberta Research Council, Alberta, Canada (non-U.S. corporation)
PI US 5550155 19960827
AI US 1994-323100 19941014 (8)
RLI Continuation of Ser. No. US 1992-914172, filed on 14 Jul 1992, now patented, Pat. No. US 5374655 which is a continuation-in-part of Ser. No. US 1992-889017, filed on 26 May 1992, now abandoned which is a continuation-in-part of Ser. No. US 1991-771259, filed on 2 Oct 1991, now abandoned which is a continuation-in-part of Ser. No. US 1991-714161, filed on 10 Jun 1991
DT Utility
EXNAM Primary Examiner: Kight, III, John; Assistant Examiner: Leary, Louise N.
LREP Burns, Doane, Swecker & Mathis
CLMN Number of Claims: 7
ECL Exemplary Claim: 1
DRWN 8 Drawing Figure(s); 8 Drawing Page(s)
LN.CNT 1837
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
AB Monofucosylated and monosialyated derivatives of the compound .beta.Gal(1-4).beta.GlcNAc(1-3).beta.Gal(1-4).beta.GlcNAc-OR, where R is hydrogen, a saccharide, an oligosaccharide or an aglycon moiety have been found to be useful in modulating a cell-mediated immune inflammatory response in mammals.

L36 ANSWER 20 OF 37 USPATFULL
AN 96:23036 USPATFULL
TI Bacterial surface protein expression
IN Smit, John, Richmond, Canada
Bingle, Wade H., Vancouver, Canada
PA The University of British Columbia, Vancouver, Canada (non-U.S. corporation)
PI US 5500353 19960319
AI US 1994-194290 19940209 (8)
RLI Continuation-in-part of Ser. No. US 1992-895367, filed on 9 Jun 1992,

now abandoned
DT Utility
EXNAM Primary Examiner: Wax, Robert A.; Assistant Examiner: Kim, Hyosuk
LREP Shlesinger, Arkwright & Garvey
CLMN Number of Claims: 5
ECL Exemplary Claim: 1
DRWN 11 Drawing Figure(s); 9 Drawing Page(s)
LN.CNT 898
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
AB This invention provides a bacterium having an S-layer modified such that the bacterium **S-layer protein** gene contains one or more in-frame sequences coding for one or more heterologous polypeptides and, the S-layer is a fusion product of the **S-layer protein** and the heterologous polypeptide. The bacterium is preferably a Caulobacter which may be cultured as a film in a bioreactor or may be used to present an antigenic epitope to the environment of the bacterium. This invention also provides a method of expressing and presenting to the environment of a Caulobacter, a polypeptide that is heterologous to the S-layer of Caulobacter which comprises cloning a coding sequence for the polypeptide in-frame into an **S-layer protein** gene of Caulobacter whereby the polypeptide is expressed and presented on the surface of the Caulobacter as a fusion product of the **S-layer protein** and the polypeptide in the S-layer of the Caulobacter.

L36 ANSWER 21 OF 37 MEDLINE
AN 96256624 MEDLINE
DN 96256624
TI Differential domain accessibility to monoclonal antibodies in three different morphological assemblies built up by the **S-layer protein** of Thermus thermophilus HB8.
AU Caston J R; Olabarria G; Lasa I; Carrascosa J L; Berenguer J
CS Centro de Biologia Molecular "Severo Ochoa" and Centro Nacional de Biotecnologia, Universidad Autonoma de Madrid, Spain.
SO JOURNAL OF BACTERIOLOGY, (1996 Jun) 178 (12) 3654-7.
Journal code: HH3. ISSN: 0021-9193.
CY United States
DT Journal; Article; (JOURNAL ARTICLE)
LA English
FS Priority Journals
EM 199610
AB A collection of 27 monoclonal antibodies (MAbs) against the **S-layer protein** (P100) of Thermus thermophilus HB8 has been obtained. They have been classified according to their ability to recognize S-layer regions expressed in E. coli from plasmids containing different fragments of its coding gene, slpA. The accessibility of the binding sites in hexagonal, trigonal, or tetragonal assemblies of P100 was analyzed by enzyme-linked immunosorbent assays with six of these MAbs and their respective Fab fragments. When packed hexagonally as the native S-layer (S1 assemblies), only a small region located near the amino terminus of the P100 was accessible. However, when P100 was assembled into trigonal (pS2 assemblies) or tetragonal (S2 assemblies) arrays, most of the protein domains analyzed were easily detected, thus suggesting that P100 is assembled in S2 and pS2 in a similar way and that these two arrangements are quite different from the S1 assembly. Relationships between accessibility and sequence predictions are discussed.

L36 ANSWER 22 OF 37 EMBASE COPYRIGHT 2000 ELSEVIER SCI. B.V.DUPLICATE 4
AN 96346348 EMBASE
DN 1996346348
TI 2-D protein crystals as an immobilization matrix for producing reaction

zones in dipstick-style immunoassays.
AU Breitwieser A.; Kupcu S.; Howorka S.; Weiger S.; Langer C.; Hoffmann-Sommergruber K.; Scheiner O.; Sleytr U.B.; Sara M.
CS ZULB, Inst. fur Molekulare Nanotechnologie, Universitat fur Bodenkultur, Gregor Mendelstrasse 33,A-1180 Vienna, Austria
SO BioTechniques, (1996) 21/5 (918-925).
ISSN: 0736-6205 CODEN: BTNQDO
CY United States
DT Journal; Article
FS 004 Microbiology
027 Biophysics, Bioengineering and Medical Instrumentation
029 Clinical Biochemistry
LA English
SL English
AB In the present study, the applicability of crystalline bacterial cell-surface layers (S-layers) as novel immobilization matrices and reaction zones for dipstick-style immunoassays was investigated. For this purpose, S-layer- carrying cell-wall fragments from Bacillus sphaericus CCM 2120 were deposited on a microporous support, and the **S-layer protein** was cross-linked with glutaraldehyde. For developing appropriate test systems, either human IgG was directly linked to the carboxylic acid groups from the **S-layer protein** or it was immobilized using **Protein A** or, after biotinylation, using **streptavidin**. A clear correlation was obtained between the amount of anti-human IgG applied and the absorbance values in the immunoassays. S-layers with covalently bound recombinant major birch pollen allergen were used for quantitative and semiquantitative determination of an antibody raised against it. Using S-layers as an immobilization matrix in comparison to amorphous polymers has advantages in that the closed monolayers of functional macromolecules on their outermost surface allows for strong signals in immunoassays, almost completely eliminates background and prevents diffusion.

L36 ANSWER 23 OF 37 MEDLINE
AN 96134966 MEDLINE
DN 96134966
TI slpM, a gene coding for an "S-layer-like array" overexpressed in S-layer mutants of Thermus thermophilus HB8.
AU Olabarria G; Fernandez-Herrero L A; Carrascosa J L; Berenguer J
CS Centro de Biologia Molecular Severo Ochoa, Consejo Superior de Investigaciones Cientificas-Universidad Autonoma de Madrid, Spain.
SO JOURNAL OF BACTERIOLOGY, (1996 Jan) 178 (2) 357-65.
Journal code: HH3. ISSN: 0021-9193.
CY United States
DT Journal; Article; (JOURNAL ARTICLE)
LA English
FS Priority Journals
OS GENBANK-X90369
EM 199604
AB S-layer deletion mutants of Thermus thermophilus HB8 overproduce a regular array which surrounds groups of several cells. Averages of two-dimensional projections revealed a detailed architecture similar in general morphology and unit cell dimensions to that of the S-layer but having a different mass distribution. The structural components of these "S-layer-like arrays" are a group of three proteins of 52 (P52), 50 (P50), and 36 (P36) kDa, which are overexpressed in S-layer mutants. These three proteins specifically bind antibodies against P52, suggesting that the smaller proteins correspond to fragments derived from P52. This hypothesis was demonstrated by the identity of the trypsin digestion products of P52 and P50. The gene slpM, responsible for the synthesis of P52, was cloned by using synthetic oligonucleotides designed from partial amino acid

sequences of P52 and P50. When slpM was expressed in Escherichia coli, proteins specifically recognized by anti-P52 antiserum whose electrophoretic mobilities were similar to those of P52 and P36 were detected. The sequence of slpM revealed the existence of an open reading frame in which the amino termini of P52, P50, and P36 were identified. The unprocessed product of slpM is a 469-amino-acid-long polypeptide whose theoretical M(r) (52,131) was in good agreement with the electrophoretic mobility of P52. The properties deduced for the product of slpM are very different from those of any **S-layer protein** so far sequenced. The possible roles of SlpM in wild-type cells are discussed.

L36 ANSWER 24 OF 37 BIOTECHDS COPYRIGHT 2000 DERWENT INFORMATION LTD
AN 1995-11949 BIOTECHDS
TI Host cell expressing surface layer protein; Bordetella pertussis P69 antigen, pertussis toxin, tetanus toxin fragment-C, Escherichia coli heat-labile toxin B-subunit or E. coli K88 antigen surface display on Bacillus sphaericus
AU Deblaere R Y; Desomer J; Dhaese P
PA Solvay
PI WO 9519371 20 Jul 1995
AI WO 1995-EP147 13 Jan 1995
PRAI GB 1994-650 14 Jan 1994
DT Patent
LA English
OS WPI: 1995-263827 [34]
AB A new host cell has a surface layer (S-layer) containing a fusion protein, composed of at least sufficient **S-layer protein** for assembly, and a heterologous protein fragment fused to the C-terminus or N-terminus, which is then presented on the outer surface of the cell. The following are also new: DNA containing a promoter (e.g. a Bacillus sp. **S-layer protein** promoter, such as the P1 promoter of Bacillus sphaericus P-1 (LMG P-13855)) operably linked to a sequence encoding a signal peptide and the fusion **protein**; a promoter with specified -35 and -10 regions; an expression vector with the promoter and a downstream cloning site; and a process for transformation of B. sphaericus P-1 by harvesting cells at late stationary phase, mixing with DNA, and carrying out electroporation. The heterologous protein may be a virus, bacterium, fungus, yeast or parasite antigen, e.g. Bordetella pertussis P69 antigen, pertussis toxin or a subunit, tetanus toxin fragment-C, Escherichia coli heat-labile toxin B-subunit or E. coli K88 antigen. Cells presenting the fusion protein on their surface may be used as a recombinant vaccine. (95pp)

L36 ANSWER 25 OF 37 EMBASE COPYRIGHT 2000 ELSEVIER SCI. B.V.DUPLICATE 5
AN 95150249 EMBASE
DN 1995150249
TI Physical and functional S-layer reconstitution in Aeromonas salmonicida.
AU Garduno R.A.; Phipps B.M.; Kay W.W.
CS Biochemistry/Microbiology Department, Canadian Bacterial Disease Network, University of Victoria, P.O. Box 3055,Victoria, BC V8W 3P6, Canada
SO Journal of Bacteriology, (1995) 177/10 (2684-2694).
ISSN: 0021-9193 CODEN: JOBAAY
CY United States
DT Journal; Article
FS 004 Microbiology
037 Drug Literature Index
LA English
SL English

AB The various functions attributed to the S-layer of Aeromonas salmonicida have been previously identified by their conspicuous absence in S-layer-defective mutants. As a different approach to establish the multifunctional nature of this S-layer, we established methods for reconstitution of the S- layer of A. salmonicida. Then we investigated the functional competence of the reconstituted S-layer. S-layers were reconstituted in different systems: on inert membranes or immobilized lipopolysaccharide (LPS) from purified **S- layer protein** (**A**-protein) or on viable cells from either A-protein or preassembled S-layer sheets. In the absence of divalent cations and LPS, purified A-protein in solution spontaneously assembled into tetrameric oligomers and, upon concentration by ultrafiltration, into macroscopic, semicrystalline sheets formed by oligomers loosely organized in a tetragonal arrangement. In the presence of Ca2+, purified A-protein assembled into normal tetragonal arrays of interlocked subunits. A-protein bound with high affinity (K(d), 1.55 x 10-7 M) and specificity to high-molecular-weight LPS from A. salmonicida but not to the LPSs of several other bacterial species. In vivo, A-protein could be reconstituted only on A. salmonicida cells which contained LPS, and Ca2+ affected both a regular tetragonal organization of the reattached A-protein and an enhanced reattachment of the A-protein to the cell surface. The reconstitution of preformed S-layer sheets (produced by an S-layer-secreting mutant) to an S-layer-negative mutant occurred consistently and efficiently when the two mutant strains were cocultured on calcium- replete solid media. Reattached A-protein (exposed on the surface of S- layer-negative mutants) was able to bind porphyrins and an S-layer-specific phage but largely lacked regular organization, as judged by its inability to bind immunoglobulins. Reattached S-layer sheets were regularly organized and imparted the properties of porphyrin binding, hydrophobicity, autoaggregation, adherence to and invasion of fish macrophages and epithelial cells, and resistance to macrophage cytotoxicity. However, cells with reconstituted S-layers were still sensitive to complement and insensitive to the antibiotics streptonigrin and chloramphenicol, indicating incomplete functional reconstitution.

L36 ANSWER 26 OF 37 USPATFULL
AN 94:110797 USPATFULL
TI Methods for the synthesis of monofucosylated oligosaccharides terminating in di-N-acetyllactosaminyl structures
IN Kashem, Mohammed, Edmonton, Canada
Venot, Andre P., Edmonton, Canada
Smith, Richard, Edmonton, Canada
PA Alberta Research Council, Edmonton, Canada (non-U.S. corporation)
PI US 5374655 19941220
AI US 1992-914172 19920714 (7)
RLI Continuation-in-part of Ser. No. US 1992-889017, filed on 26 May 1992 which is a continuation-in-part of Ser. No. US 1991-771259, filed on 2 Oct 1991, now abandoned which is a continuation-in-part of Ser. No. US 1991-714161, filed on 10 Jun 1991
PRAI WO 1992-251 19920610
DT Utility
EXNAM Primary Examiner: Russel, Jeffrey E.; Assistant Examiner: Leary, Louise N.
LREP Burns, Doane, Swecker & Mathis
CLMN Number of Claims: 10
ECL Exemplary Claim: 1
DRWN 8 Drawing Figure(s); 8 Drawing Page(s)
LN.CNT 2027
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
AB Disclosed are methods for the preparation of monofucosylated and

sialylated derivatives of the compound .beta.Gal(1-4).beta.GlcNAc(1-3).beta.Gal(1-4).beta.GlcNAc-OR. In particular, the methods of this invention provide for a multi-step synthesis wherein selective monofucosylation is accomplished on the 3-hydroxy group on only one of the GlcNAc units found in the .beta.Gal(1-4) .beta.GlcNAc (1-3) .beta.Gal (1-4) .beta.GlcNAc-OR compound. In this step, monofucosylation is achieved by use of the .alpha.(1-3)fucosyltransferase.

L36 ANSWER 27 OF 37 MEDLINE
AN 95035101 MEDLINE
DN 95035101
TI An archaeal S-layer gene homolog with repetitive subunits.
AU Yao R; Macario A J; Conway de Macario E
CS Wadsworth Center for Laboratories and Research, New York State Department of Health, Albany..
SO BIOCHIMICA ET BIOPHYSICA ACTA, (1994 Nov 22) 1219 (3) 697-700.
Journal code: A0W. ISSN: 0006-3002.
CY Netherlands
DT Journal; Article; (JOURNAL ARTICLE)
LA English
FS Priority Journals; Cancer Journals
OS GENBANK-X77929; GENBANK-X58297; GENBANK-X58296; GENBANK-M59200; GENBANK-M62816
EM 199502
AB An **S-layer protein** gene homolog of the slgA gene of two Methanothermus species was found in the genome of another methanogenic archaeon of a different family, Methanosarcina mazei S-6. The new gene (slgB) encodes a molecule (SlpB) with the characteristics of S-layer proteins. The N-terminal half of SlpB is 44% identical to that encoded by SlgA, but the other half shows distinctive features: four 56 amino acid long tandem repeats, and Trp-Xaa-Trp clusters located six amino acids from the N-terminus of each repeat.

L36 ANSWER 28 OF 37 EMBASE COPYRIGHT 2000 ELSEVIER SCI. B.V.DUPLICATE 6
AN 94059473 EMBASE
DN 1994059473
TI Novel **Protein A** affinity matrix prepared from two-dimensional protein crystals.
AU Weiner C.; Sara M.; Sleytr U.B.
CS Zentrum fur Ultrastrukturforschung, Institut Molekulare Nanotechnologie, Universitat fur Bodenkultur, Gregor Mendel Strasse 33,A-1180 Vienna, Austria
SO Biotechnology and Bioengineering, (1994) 43/4 (321-330).
ISSN: 0006-3592 CODEN: BIBIAU
CY United States
DT Journal; Article
FS 029 Clinical Biochemistry
LA English
SL English
AB In this article, we describe a novel type of affinity matrix which was prepared by covalently binding **Protein A** to crystalline cell surface layers (S-layers) from the gram-positive Clostridium thermohydrosulfuricum L111-69. S-layers were used in the form of cell wall fragments, which were obtained by breaking whole cells by ultrasonification and removing the cell content and the plasma membrane. In these thimble-shaped structures, revealing a size of 1 to 2 .mu.m, the peptidoglycan-containing layer was covered on both faces with a hexagonally ordered S-layer lattice composed of identical glycoprotein subunits. After crosslinking the **S-layer protein** with glutaraldehyde, carboxyl groups from acidic amino acids were activated with carbodiimide and used for immobilization of **Protein A**. Quantitative determination confirmed that up

to two **Protein A** molecules were bound per S-layer subunit leading to a dense monomolecular coverage of the immobilization matrix with the ligand. Affinity microparticles were capable of adsorbing IgG from solutions of purified preparations, from artificial IgG-albumin mixtures, and from serum. The amount of IgG bound to affinity microparticles corresponded to the theoretical saturation capacity. Under appropriate conditions, up to 95% of the adsorbed IgG could be eluted again. Affinity microparticles were found to have an extremely low **Protein A** leakage and a high stability toward mechanical forces. Because pores in the S-layer lattice revealed a size of 4 to 5 nm, immobilization of **Protein A** and adsorption of IgG was restricted to the outermost surface area. This excludes mass transfer problems usually encountered with affinity matrices prepared from amorphous polymers where more than 90% of the ligands are immobilized in the interior.

L36 ANSWER 29 OF 37 EMBASE COPYRIGHT 2000 ELSEVIER SCI. B.V.DUPLICATE 7
AN 94171145 EMBASE
DN 1994171145
TI Affinity cross-flow filtration: Purification of IgG with a novel **protein a** affinity matrix prepared from two-dimensional protein crystals.
AU Weiner C.; Sara M.; Dasgupta G.; Sleytr U.B.
CS Zentrum fur Ultrastruckturforschung, L. Boltzmann-Inst Molek Nanotechnol., Universitat fur Bodenkultur, Gregor Mendel Strasse 33,A-1180 Vienna, Austria
SO Biotechnology and Bioengineering, (1994) 44/1 (55-65). ISSN: 0006-3592 CODEN: BIBIAU
CY United States
DT Journal; Article
FS 004 Microbiology
029 Clinical Biochemistry
LA English
SL English
AB In this article, we describe the use of 1- to 2-.mu.m sized affinity microparticles for the isolation and purification of IgG from artificial IgG-human serum albumin mixtures and clarified hybridoma cell culture supernatants by affinity cross-flow filtration. Affinity microparticles were prepared from cell wall fragments of Clostridium thermohydrosulfuricum L111-69, in which the peptidoglycan-containing layer was completely covered with a hexagonally ordered S-layer lattice. After crosslinking the **S-layer protein** with glutaraldehyde, carboxyl groups from acidic amino acids were activated with carbodiimide and used for immobilization of **Protein A**. Quantitative determination confirmed that **Protein A** molecules formed a monomolecular layer on the outermost surface of the S-layer lattice. Affinity microparticles were found to withstand high centrifugal and shear forces and revealed no **Protein A** leakage or **S-layer protein** release under cross-flow conditions between pH 2 to 12. The IgG-binding capacity of affinity microparticles was investigated under cross-flow conditions and compared with that obtained in batch adsorption processes.

L36 ANSWER 30 OF 37 BIOSIS COPYRIGHT 2000 BIOSIS
AN 1993:432281 BIOSIS
DN PREV199396086906
TI Rearrangement of sapA homologs with conserved and variable regions in Campylobacter fetus.
AU Tummuru, Murali K. R.; Blaser, Martin J. (1)
CS (1) Vanderbilt University Sch. Med., Div. Infectious Diseases, A-3310 Med. Center North, Nashville, TN 37232-2605 USA

SO Proceedings of the National Academy of Sciences of the United States of America, (1993) Vol. 90, No. 15, pp. 7265-7269.
ISSN: 0027-8424.
DT Article
LA English
AB The Campylobacter fetus surface-layer (Slayer) proteins mediate both complement resistance and antigenic variation in mammalian hosts. Wild-type strain 23D possesses the sapA gene, which encodes a 97-kDa Slayer protein, and several sapA homologs are present in both wildtype and mutant strains. Here we report that a cloned silent gene (sapA1) in C. fetus can express a functional full-length **S-layer protein** in Escherichia coli. Analysis of sapA and sapA1 and partial analysis of sapA2 indicate that a block of apprxeq 600 bp beginning upstream and continuing into the open reading frames is completely conserved, and then the sequences diverge completely, but immediately downstream of each gene is another conserved 50-bp sequence. Conservation of sapA1 among strains, the presence of a putative Chi (RecBCD recognition) site upstream of sapA, sapA1, and sapA2, and the sequence identities of the sapA genes suggest a system for homologous recombination. Comparison of the wild-type strain (23D) with a phenotypic variant (23D-11) indicates that variation is associated with removal of the divergent region of sapA from the expression locus and exchange with a corresponding region from a sapA homolog. We propose that site-specific reciprocal recombination between sapA homologs leads to expression of divergent S-layer proteins as one of the mechanisms that C. fetus uses for antigenic variation.

L36 ANSWER 31 OF 37 BIOSIS COPYRIGHT 2000 BIOSIS
AN 1993:479464 BIOSIS
DN PREV199396113064
TI Aeromonas salmonicida grown in vivo.
AU Garduno, Rafael A.; Thornton, Julian C.; Kay, William W. (1)
CS (1) Canadian Bacterial Disease Network, Univ. Victoria, P.O. Box 3055, Victoria, British Columbia V8W 3P6 Canada
SO Infection and Immunity, (1993) Vol. 61, No. 9, pp. 3854-3862.
ISSN: 0019-9567.
DT Article
LA English
AB The virulent fish pathogen Aeromonas salmonicida was rapidly killed in vivo when restricted inside a diffusion chamber implanted intraperitoneally in rainbow trout. After a period of regrowth, the survivors had acquired resistance to host-mediated bacteriolysis, phagocytosis, and oxidative killing, properties which were subsequently lost by growth in vitro. Resistance to bacteriolysis and phagocytosis was associated with a newly acquired capsular layer revealed by acidic polysaccharide staining and electron microscopy. This capsular layer shielded the underlying, regular surface array (S-layer) from immunogold labeling with a primary antibody to the **S-layer protein**. Resistance to oxidative killing was mediated by a mechanism not associated with the presence of the capsular layer. An attenuated vaccine strain of A. salmonicida grown in vivo failed to express the capsular layer. Consequently, the in vivo-grown cells of this attenuated strain remained as sensitive to bacteriolysis, and as avidly adherent to macrophages, as the in vitro-grown cells. The importance of these new virulence determinants and their relation to the known virulence factors of A. salmonicida are discussed.

L36 ANSWER 32 OF 37 MEDLINE
AN 93054364 MEDLINE
DN 93054364
TI Sequence of the S-layer gene of Thermus thermophilus HB8 and functionality

of its promoter in Escherichia coli.
AU Faraldo M M; de Pedro M A; Berenguer J
CS Centro de Biologia Molecular, Universidad Autonoma de Madrid-Consejo Superior de Investigaciones Cientificas, Spain..
SO JOURNAL OF BACTERIOLOGY, (1992 Nov) 174 (22) 7458-62.
Journal code: HH3. ISSN: 0021-9193.
CY United States
DT Journal; Article; (JOURNAL ARTICLE)
LA English
FS Priority Journals
OS GENBANK-X57333; GENBANK-D10158; GENBANK-Z11768; GENBANK-L01135; GENBANK-L01136; GENBANK-L01137; GENBANK-L01138; GENBANK-L01139; GENBANK-L01140; GENBANK-L01141
EM 199302
AB The nucleotide sequence of the slpA gene, which is responsible for the synthesis of the **S-layer protein** of Thermus thermophilus HB8, is described. This gene is transcribed as a unit in which the coding region is preceded by a 127-base-long leader mRNA sequence. The promoter region is also recognized by the RNA polymerase of Escherichia coli because of the presence of homologous -35 and -10 boxes. Homologies with other promoters from Thermus spp. are also presented.

L36 ANSWER 33 OF 37 CAPLUS COPYRIGHT 2000 ACS
AN 1993:229810 CAPLUS
DN 118:229810
TI Novel structural patterns in divalent cation-depleted surface layers of Aeromonas salmonicida
AU Garduno, Rafael A.; Phipps, Barry M.; Baumeister, Wolfgang; Kay, William W.
CS Dep. Biochem. Microbiol., Univ. Victoria, Victoria, BC, V8W 3P6, Can.
SO J. Struct. Biol. (1992), 109(3), 184-95
CODEN: JSBIEM; ISSN: 1047-8477
DT Journal
LA English
AB The fish pathogen A. salmonicida possesses a regular surface layer (or A-layer) which is an important virulence determinant. The A-**protein**, a single bilobed protein organized in a p4 lattice of M4C4 arrangement with 2 morphol. domains, comprises this layer.
The role of divalent cations in the A-layer structure was studied to better understand A-protein subunit interactions affecting structural flexibility and function. Divalent cation bridges were found to be involved in the integrity of the A-layer. Two novel A-layer patterns were formed as the result of growth under Ca limitation or by chelation of divalent cations with EDTA or EGTA, thereby constituting the 1st reported case of formation of distinct regular arrays upon divalent cation depletion. Under these conditions A-protein was sometimes released as tetrameric units, rather than in monomeric form. The formation of the 2 novel patterns is best explained by a sequence of structural rearrangements, following disruption of only 1 of the 2 A-layer morphol. units, i.e., those held together by divalent cation bridges. The free tetrameric units represent 4 A-protein subunits clustered around the unaffected 4-fold axis.

L36 ANSWER 34 OF 37 MEDLINE DUPLICATE 8
AN 89327128 MEDLINE
DN 89327128
TI Cloning and sequencing of the gene encoding a 125-kilodalton surface-layer protein from Bacillus sphaericus 2362 and of a related cryptic gene.
AU Bowditch R D; Baumann P; Yousten A A
CS Department of Microbiology, University of California, Davis 95616..
SO JOURNAL OF BACTERIOLOGY, (1989 Aug) 171 (8) 4178-88.
Journal code: HH3. ISSN: 0021-9193.

CY United States
DT Journal; Article; (JOURNAL ARTICLE)
LA English
FS Priority Journals
OS GENBANK-M28361
EM 198911
AB Using the vector pGEM-4-blue, a 4,251-base-pair DNA fragment containing the gene for the surface (S)-**layer protein** of Bacillus sphaericus 2362 was cloned into Escherichia coli. Determination of the nucleotide sequence indicated an open reading frame (ORF) coding for a protein of 1,176 amino acids with a molecular size of 125 kilodaltons (kDa). A protein of this size which reacted with antibody to the 122-kDa **S-layer protein** of B. sphaericus was detected in cells of E. coli containing the recombinant plasmid. Analysis of the deduced amino acid sequence indicated a highly hydrophobic N-terminal region which had the characteristics of a leader peptide. The first amino acid of the N-terminal sequence of the 122-kDa **S-layer protein** followed the predicted cleavage site of the leader peptide in the 125-kDa **protein**. **A** sequence characteristic of promoters expressed during vegetative growth was found within a 177-base-pair region upstream from the ORF coding for the 125-kDa protein. This putative promoter may account for the expression of this gene during the vegetative growth of B. sphaericus and E. coli. The gene for the 125-kDa protein was followed by an inverted repeat characteristic of terminators. Downstream from this gene (11.2 kilobases) was an ORF coding for a putative 80-kDa protein having a high sequence similarity to the 125-kDa protein. Evidence was presented indicating that this gene is cryptic.

L36 ANSWER 35 OF 37 MEDLINE DUPLICATE 9
AN 88115139 MEDLINE
DN 88115139
TI Surface protein composition of Aeromonas hydrophila strains virulent for fish: identification of a surface array protein.
AU Dooley J S; Trust T J
CS Department of Biochemistry and Microbiology, University of Victoria, British Columbia, Canada..
SO JOURNAL OF BACTERIOLOGY, (1988 Feb) 170 (2) 499-506.
Journal code: HH3. ISSN: 0021-9193.
CY United States
DT Journal; Article; (JOURNAL ARTICLE)
LA English
FS Priority Journals
EM 198805
AB The surface protein composition of members of a serogroup of Aeromonas hydrophila which exhibit high virulence for fish was examined. Treatment of whole cells of representative strain A. hydrophila TF7 with 0.2 M glycine buffer (pH 4.0) resulted in the release of sheets of a tetragonal surface protein array. Sodium dodecyl sulfate (SDS)-polyacrylamide gel electrophoresis analysis showed that this sheet material was composed primarily of a protein of apparent molecular weight 52,000 (52K **protein**). **A** 52K protein was also the predominant protein in glycine extracts of other members of the high-virulence serogroup. Immunoblotting with antiserum raised against formalinized whole cells of A. hydrophila TF7 showed the 52K **S-layer protein** to be the major surface protein antigen, and impermeant Sulfo-NHS-Biotin cell surface labeling showed that the 52K **S-layer protein** was the only protein accessible to the Sulfo-NHS-Biotin label and effectively masked underlying outer membrane (OM) proteins. In its native surface conformation the 52K **S-layer protein** was only weakly reactive with a lactoperoxidase 125I surface iodination procedure. A UV-induced rough lipopolysaccharide (LPS) mutant of TF7 was found to produce an intact S layer, but a deep rough LPS mutant was unable to maintain an array on the

cell surface and excreted the **S-layer protein** into the growth medium, indicating that a minimum LPS oligosaccharide size was required for A. hydrophila S-layer anchoring. (ABSTRACT TRUNCATED AT 250 WORDS)

L36 ANSWER 36 OF 37 MEDLINE DUPLICATE 10
AN 88268351 MEDLINE
DN 88268351
TI Localized insertion of new S-layer during growth of Bacillus stearothermophilus strains.
AU Gruber K; Sleytr U B
CS Zentrum fur Ultrastrukturforschung, Universitat fur Bodenkultur, Wien, Austria..
SO ARCHIVES OF MICROBIOLOGY, (1988) 149 (6) 485-91.
Journal code: 7YN. ISSN: 0302-8933.
CY GERMANY, WEST: Germany, Federal Republic of
DT Journal; Article; (JOURNAL ARTICLE)
LA English
FS Priority Journals
EM 198810
AB Bacillus stearothermophilus strains PV 72 and ATCC 12980 carry a crystalline surface layer (S-layer) with hexagonal (p6) and oblique (p2) symmetry, respectively. Sites of insertions of new subunits into the regular lattice during cell growth have been determined by the indirect fluorescent antibody technique and the **protein** A /colloidal gold technique. During S-layer growth on both bacillus strains the following common features were noted: 1. shedding of intact S-layer or turnover of individual subunits was not seen; 2. new S-layer was deposited in helically-arranged bands over the cylindrical surface of the cell at a pitch angle related to the orientation of the lattice vectors of the crystalline array; 3. little or no S-layer was inserted into pre-existing S-layer at the poles, and 4. septal regions and, subsequently, newly formed cell poles were covered with new **S-layer protein.**

L36 ANSWER 37 OF 37 BIOSIS COPYRIGHT 2000 BIOSIS DUPLICATE 11
AN 1987:317608 BIOSIS
DN BA84:37115
TI THE CELL ENVELOPE OF THERMOPROTEUS-TENAX THREE-DIMENSIONAL STRUCTURE OF THE SURFACE LAYER AND ITS ROLE IN SHAPE MAINTENANCE.
AU WILDHABER I; BAUMEISTER W
CS MAX-PLANCK-INST. BIOCHEM., D-8033 MARTINSRIED BEI MUENCHEN, FRG.
SO EMBO (EUR MOL BIOL ORGAN) J, (1987) 6 (5), 1475-1480.
CODEN: EMJODG. ISSN: 0261-4189.
FS BA; OLD
LA English
AB The sulphur-dependent archaebacterium Thermoproteus tenax has a cylindrical cell shape variable in length, but constant in diameter. Its whole surface is covered by a regular protein layer (S-layer). The lattice has p6 symmetry and a lattice constant of 32.8 nm. The three-dimensional reconstruction from a tilt series of isolated and negatively stained S-layer shows a complex mass distribution of the **protein**: a prominent, pillar-shaped protrusion is located at the 6-fold crystallographic axis with radiating arms connecting neighbouring hexamers in the vicinity of the 3-fold axis. The base vectors of the S-layer lattice have a preferred orientation with respect to the longitudinal axis of the cell. The layer can be seen as a helical structure consisting of a right-handed, two-stranded helix, with the individual chains running parallel. Supposing that new **S-layer protein**

is inserted at lattice faults (wedge disclinations) near the poles, growing of the layer would then proceed by moving a disclination at the end of the helix. The constant shape of the cell, as well as the particular structure of the layer, strongly suggest that this S-layer has a shape-maintaining function.